GENOME MAPPING RESEARCH - ANIMALS

A Directory of USDA and State Projects in CRIS

Prepared by

Current Research Information System
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and

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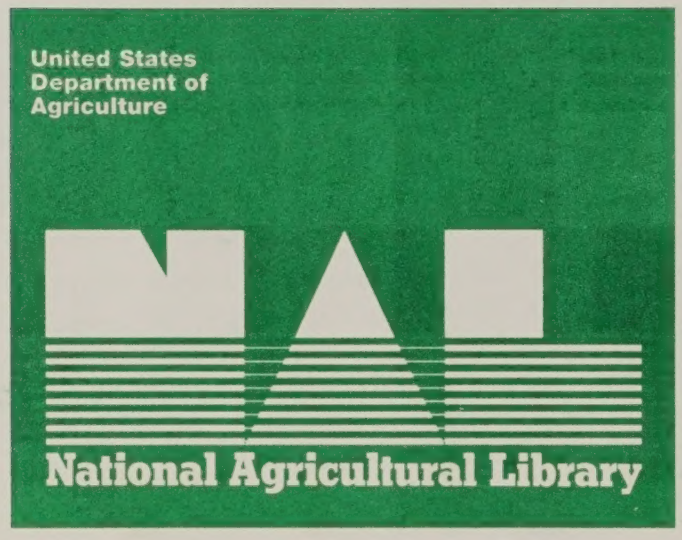
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PREFACE

This directory is a selected listing of research projects dealing with genome mapping in major animal commodities. The source for all projects was the Current Research Information System (CRIS), the research project documentation and reporting system of the U.S. Department of Agriculture (USDA).

The CRIS system contains projects conducted or sponsored by the USDA research agencies, the State agricultural experiment stations and land-grant institutions, U.S. schools and colleges of veterinary medicine, and other cooperating State institutions. Subject coverage includes the area of agriculture and forestry, in all its broad aspects, as well as food and human nutrition, environmental quality, and rural development. CRIS is operated by the Cooperative State Research Service, USDA.

Projects in this directory were retrieved from the base of projects in CRIS as of September 22, 1989. From the total of 1,845 projects initially retrieved on genome mapping, 860 were judged to have 50% or greater relevance to the topic. Distribution within the 860 projects for the plant, animal and other commodity groupings is as follows:

Commodity	No. of Projects*
Plants	427
Animals	255
Other	223

*includes overlap

Individual research directories were prepared based on this distribution, with titles reflecting the particular content of each directory.

Full project abstracts appear in this directory in the main entry section entitled, "Research Project Descriptions." The section is divided into chapters based on commodity classifications used for classifying projects in the CRIS system. Abstracts appear in chapters that correspond to the commodity classifications assigned when the projects were submitted for entry in CRIS. Projects assigned more than one commodity classification are repeated in each of the applicable chapters. Repeat entries are identified by an asterisk next to the directory number in the main entry section and in the indexes.

Arrangement of projects within chapters is alphabetical by State or country, followed in order by name of performing institution, department, and principal investigator.

The three indexes provided are the Keyword/Title Index, Investigator Index, and Performing Institution Index. Directory numbers in the indexes are used for locating projects in the main entry section. The two left-most digits correspond to the chapter in which the project is located, and the three right-most digits indicate its position within the chapter.

Index terms in the Keyword/Title Index are the keywords originally assigned by CRIS primarily for use in computer retrieval. Project titles have been inserted to provide context.

The Investigator Index is an alphabetical listing of the principal and co-investigators on the projects.

The Performing Institution Index lists the names of the institutions alphabetically by city within the State or country.

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To obtain abstracts of newer projects or for later updates of progress and publications on projects listed here, users may directly access the CRIS online file, CRIS/USDA (File 60), on DIALOG, the online retrieval system of DIALOG Information Services, Inc. The CRIS accession number shown opposite the directory number in the main entry section may be used with a TYPE or DISPLAY command on DIALOG to retrieve project information online at the user's terminal. Format 7 in the online file carries the narrative summary including the most recent update of progress and publication citations entered on the project. Projects remain on the file for 3-4 years beyond their termination date and are then purged. The file is updated monthly.

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This directory was compiled by Philip L. Dopkowski and F. Allen Moore, Technical Products and Services, CRIS. Edward A. Warnick, Information Systems Division, National Agricultural Library, provided technical assistance. Dr. Bruno Quebedeaux, Cooperative State Research Service, provided scientific oversight and support in the review and assessment of projects for relevancy.

RESEARCH PROJECT DESCRIPTIONS

CM 08 FISH AND WILDLIFE

08.001 CRIS0130506
MOLECULAR BIOLOGY OF CHANNEL CATFISH VIRUS PATHOGENICITY

NUSBAUM K E; GRIZZLE J M; BIRD R C;
Microbiology; Auburn University, Auburn, **ALABAMA** 36830.
Proj. No.: ALAV-0174 Project Type: STATE
Agency ID: CSVM Period: 01 OCT 85 to 30 SEP 89

Objectives: Determine the genetic, biochemical, and antigenic structures of six isolates of CCV.

Approach: Six isolates of CCV are being fingerprinted through the use of restriction endonuclease and agarose gel electrophoresis. Envelope proteins are examined by detergent lysis of virus and are then electrophoresed in polyacrylamide gel. Antigenicity of the isolates will be compared by viral neutralization kinetics. Viral replication and dissemination in the host will be monitored through the use of 9 recombinant DNA probes which have been cloned from the CCV genone and have been shown to encode several genes expressed during infection.

Progress: 87/10 to 88/09. Channel catfish virus (CCV) gene expression has been elicited 6-8 hours after seining fingerlings from ponds. Gene expression activity quickly subsides by 24th hour after removal of stressors. Virus could not be isolated from liver or kidney of stressed fish. A viral envelope vaccine has proven efficacious in preventing death due to CCV infection 2 months after bath immersion. Clone pCCV3E14 has been partially sequenced in an effort to understand expression in the late phases of replication.

Publications: 87/10 to 88/09
BIRD, R.C., NUSBAUM, K.E., SCREWS, E.A., YOUNG-WHITE, R.R., GRIZZLE, J.M. and TOIVIO-KINNUCAN, M. 1988. Molecular cloning of fragments of the channel catfish virus (Herpesviridae) genome and exp. of the encoded mRNAs during infection.

08.002 CRIS0138576
IMMUNOLOGY AND MOLECULAR BIOLOGY OF ECTOPARASITIC MITES

BOYCE W M; Veterinary Microbiology Immuno; University of California, Davis, **CALIFORNIA** 95616.
Proj. No.: CA-V*-VMB-5093-H Project Type: HATCH - PENDING
Agency ID: CSRS Period: 11 JUL 89 to 30 SEP 93

Objectives: To develop an immunoassay for diagnosis of mite infestations, determine the genetic relatedness of mites using molecular analysis of parasite DNA; and conduct epidemiologic studies of host specificity and treatment strategies.

Approach: Immunoassays will be developed using antigens extracted from mites and sera from naturally and experimentally infected animals. Cross transmission studies will be performed by transferring mites onto new hosts under experimental conditions. Genetic relatedness will be determined through a variety of molecular biology techniques including RFLP and DNA hybridization.

08.003 CRIS0077856
IMMUNOLOGY AND PATHOGENESIS OF PARASITIC DISEASES OF ANIMALS

KAZACOS K R; Veterinary Pathobiology; Purdue University, West Lafayette, **INDIANA** 47907.
Proj. No.: IND073029 Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 88 to 30 SEP 93

Objectives: For selected naturally occurring parasitic diseases of animals, determine: Immune responses, mechanisms, and immunodiagnosis. Pathogenesis, pathology, and parasitologic parameters. Prevalence and epidemiologic patterns. Focus these studies on the following diseases, and others as they might arise: Ascariasis (Ascaris suum) of swine. Trichinellosis (Trichinella spiralis of swine and wildlife. Nematode larva migrans diseases of animals (Baylisascaris, Tococara).

Approach: Naturally occurring and experimentally induced cases of selected parasitic diseases will be studied by laboratory and field observatios and methods, including the application of modern immunochemical and molecular biologic techniques.

Progress: 87/10 to 88/09. Twenty-one wildlife isolates of Trichinella spiralis were analyzed using DNA restriction fragment length polymorphisms (RFLPs). Similar RFLP patterns were seen with enzymes Eco RI, Hae III, Hpa II, Hind III and Xba I. Cla I digestion produced unique RFLPs indicating that the sylvatic group is a heterogeneous complex. Swine Trichinella

*REPEAT ENTRY

RFLP was different from sylvatic Trichinella RFLPs. RFLP analysis and Southern blot hybridizations using a U.S.D.A. swine Trichinella-specific DNA probe confirmed that one coyote isolate actually represented sylvatically-maintained swine Trichinella. This has important implications for the epidemiology of swine trichinosis. Research on the immunodiagnosis of Baylisascaris larva migrans indicated the close relationship between B. melis and B. procyonis, based on gradient SDS-PAGE of larval excretory-secretory (ES) proteins. B. columnaris ES was less like the other two, and B. transfuga ES was most dissimilar. Carbohydrate staining and biotinylated lectin binding on Western blots indicated that B. procyonis ES antigens were complex glycoproteins with many sugars represented. Immunologic cross-reactivity was directed primarily at carbohydrate epitopes. Work was initiated to produce and characterize monoclonal antibodies against B. procyonis ES. Other research indicated that a portable flame gun is potentially useful for decreasing transmission of the swine roundworm Ascaris suum in growing-finishing swine units, through destruction of the resistant eggs.

Publications: 87/10 to 88/09

BOYCE, W.M., KAZACOS, E.O., KAZACOS, K.R. and ENGELHARDT, J.A. (1987). Pathology of pentastomid infections (Sebekia mississippiensis) in fish. J. Wildl. Dis. 23:689-692.

BOYCE, W.M., BRANSTETTER, B.A. and KAZACOS, K.R. (1988). In vitro culture of Baylisascaris procyonis and initial analysis of larval excretory-secretory antigens. Proc. Helminthol. Soc. Wash. 55:15-18.

KAZACOS, K.R. and KAZACOS, E.A. (1988). Diagnostic exercise: Neuromuscular condition in rabbits. Lab. Anim. Sci. 38:187-189.

BOYCE, W.M., BRANSTETTER, B.A., and KAZACOS, K.R. (1988). Comparative analysis ot larval excretory-secretory antigens of Baylisascaris procyonis, Toxocara canis and Ascaris suum by western blotting and enzyme immunoassay.

DIXON, D., REINHARD, G.R., KAZACOS, K.R., and ARRIAGA, C. (1988). Cerebrospinal nematodiasis in prairie dogs from a research facility. J. Am. Vet. Med. Assoc. 193:251-253.

HAMANN, K.J., KEPHART, G.M., KAZACOS, K.R., and GLEICH, G.J. Immunofluorescent localization of eosinophil granule major basic protein in fatal human cases of Baylisascaris procyonis infection. Am. J. Trop. Med. Hyg,. In press.

LITTLE, A.S. (1987). Immunological comparison of larval Baylisascaris procyonis, Toxocara canis, and Ascaris suum using immunodiffusion. M.S. Thesis, Purdue University, West Lafayette. 101 p.

08.004 CRIS0131959

BIOLOGY AND CONTROL OF AQUATIC ANIMAL DISEASES

THUNE R L; JOHNSON M C; HANSON L; Veterinary Science; Louisiana State University, Baton Rouge, **LOUISIANA** 70803.

Proj. No.: LABO2607 Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 87 to 30 SEP 92

Objectives: To develop immunological procedures for studying, diagnosing and preventing aquatic animal diseases. To examine the structure, biology and pathology of aquatic animal disease organisms.

Approach: The CCV TK gene will be identified in restriction enzyme digests of the CCV genome either by using a P labeled oligonucleotide probe or by TK transformation of TK culture cells. If the probe hybridizes with CCV DNA, the genome will be cut with restriction enzymes and analyzed by Southern hybridization. If the oligonucleotide probe method does not work due to non-homology, the TK gene will be located by identifying the restriction enzyme fragment that transfects TK channel catfish/ovary (CCO) cells making them TK . Once identified, the restriction fragment containing the TK gene will be cloned into a plasmid, and amplified in E. coli. The antigenic structure of the cell wall of several strains of Edwardsiella ictaluri will be compared. Purified outer membrane proteins will be separated by sodium dodecyl sulfate polyactylamide-gel electrophoresis (SDS-PAGE) and the antigens which are recognized by channel catfish during an infection will be identified by trans-blotting outer membrane proteins and LPS and staining with an enzyme linked immunosorbant assay (ELISA).

Progress: 88/01 to 88/12. A TK deficient channel catfish ovary cell line (CCOBr) was produced by culturing CCO cells in increasing amounts of 5'-bromo2'-deoxyuridine (BUdR). This cell line exhibited low TK activity in comparison to its parent CCO cell line. Infection of the CCOBr line with CCV resulted in increased amounts of TK activity indicating that CCV coded for this enzyme. CCV was shown to be sensitive to the TK activated antiherpetic agent 1-B-D arabinofuranosl-thymine (Ara-T). An Ara-T resistant mutant of CCV was selected (CCVAr) and was shown to lack the TK inducing ability of the wild-type virus. Extracts from CCO cells and CCV infected CCOBr cells were used to characterize the cellular and viral TK isozymes. A CCV genomic library was developed by cloning DNA from purified virus into plasmid phC19 and cosmid pHC79. These clones fragments are being used in marker-rescue experiments to map the TK gene on the CCV genome. The selective medium of Fijan (1969) was modified by varying levels of Neomycin and Polymyxin B alone or in combination in Cytophaga medium. A formulation containing 4 ug/ml Neomycin and 200 units/ml Polymyxin B was most effective in enhancing detection of F. columnaris in mixed cultures. Use of the selective medium in 67 cases submitted to the Louisiana Aquatic Animal Disease Lab has resulted in a four fold increase in the isolation of F. columnaris from systemic infections.

Publications: 88/01 to 88/12
HANSON, L.A. and THUNE, R.L. 1988. Development and partial characterization of thymidine kinase deficient strain of the channel catfish ovary cell line. International Fish Health Meeting, Vancouver, B.C. Canada. p. 170.
HAWKE, J.P. and THUNE, R.L. 1988. Evaluation of a medium for the selective isolation of Flexibacter columnaris from diseased channel catfish. International Fish Health Meeting, Vancouver, B.C., Canada. p. 87.
VOGELBEIN, W. and THUNE, R.L. 1988. Ultrastructural features of three ectocommensal ectocommensal protozoa attached to the gills of the redswamp crawfish, Procambarus clarkii. Journal of Protozoology 35(3):337-344.

08.005 CRIS0088280
RAPID IDENTIFICATION OF CHANNEL CATFISH VIRUS CARRIERS

BOYLE J A; Biochemistry; Mississippi State University, Mississippi State, **MISSISSIPPI** 39762.
Proj. No.: MIS-0868 Project Type: SPECIAL GRANT
Agency ID: CSRS Period: 15 SEP 82 to 31 MAR 85

Objectives: The aim of the research is to develop a method to identify brood stock catfish that are carriers of channel catfish virus. The method will then be used to study the mode of transmission of the virus.

Approach: DNA isolated from catfish virus will be used as a hybridization probe to search for the presence of similar DNA sequences in catfish. The sequences could be from viral sequences integrated into the catfish genome or from viral DNA or whole viruses present in the catfish eggs.

Progress: 85/01 to 85/12. No progress reported this period.

Publications: 85/01 to 85/12
WISE, J.A., BOWSER, P. and BOYLE, J.A. 1985. Detection of channel catfish virus in asymptomatic adult fish. J. Fish Dis. (in press).
COLYER, T.E. and BOYLE, J.A. 1985. Optimization of conditions for production of channel catfish ovary cells and channel catfish virus DNA. Appl. Environ. Microbiol. 49: 1025-1028.
WISE, J.A. and BOYLE, J.A. 1985. Detection of channel catfish virus in channel catfish: use of a nucleic acid probe. J. Fish Dis. 8: 417-424.

08.006 CRIS0097889
COLONY STRUCTURE OF THE NAKED MOLE-RAT: A EUSOCIAL MAMMAL

SHERMAN P W; AQUADRO C F; Neurobiology and Behavior; Cornell University, Ithaca, **NEW YORK** 14853.
Proj. No.: NYC-191421 Project Type: HATCH
Agency ID: CSRS Period: 12 FEB 86 to 30 SEP 89

Objectives: I will investigate the behavioral and genetical foundations of colony structure in the naked mole-rat (Heterocephalus glaber), a highly social, subterranean rodent. Regarding behavior, I will attempt to answer three questions: How does each individual's participation in maintenance and defense of its colony change as it ages? Do individual mole-rats specialize on particular maintenance or defense activities? What is the role of the single breeding female in organizing and regulating colony activity? Regarding the genetics of colony structure, I will investigate what is the average genetic relatedness within and among colonies in the field, and what is the mating system, and which male or males sire pups in each litter. Through this research, I intend to develop naked mole-rats as a "model" system for exploring the factors affecting conflict and cooperation in the social behavior of other wild and domestic animals.

Approach: The research will involve (1) detailed behavioral observations on individually marked mole-rats in my laboratory, and (2) determining genetic relatedness within and among colonies in the field (and lab) through restriction endonuclease analyses of the animals' nuclear and mitochondrial DNA. The biochemical studies will be done in collaboration with Prof. C.F. Aquadro of Cornell's Section of Genetics and Development.

Progress: 87/11 to 88/10. DNA probes derived from tandem-repetitive sequences in humans and the M13 phage have revealed repeat sequence length variants throughout the genomes of a variety of vertebrates so polymorphic that they can generate DNA "fingerprints", useful for assigning paternity and maternity in humans and birds. We used three probes that can detect different families of minisatellites, two derived from the human myoglobin gene and another from wild-type M13 phage DNA, to investigate the genetic structure within and among colonies of a eusocial mammal, the naked mole-rat (Heterocephalus glaber). DNA fingerprints of individuals from four wild-caught colonies and their descendants were strikingly similar. Fingerprints of colony-mates were almost identical, and between colonies they were much more similar than fingerprints of unrelated individuals in all other species of free-living vertebrates. Extreme genetic homogeneity within naked mole-rat colonies was due to close genetic relationship, itself the result of consanguineous mating. This is the highest F value ever recorded among wild mammals. The genetic stucture of naked mole-rat colonies can now be interpreted in light of kin selection and ecological constraints model for the evolution of cooperative breeding and eusociality. Apparently the patchiness of the animals' food resources and advantages of group defense against predators have favored individuals that remain in the natal colony rather than dispersing.

Publications: 87/11 to 88/10
REEVE, H.K., WESTNEAT, D.F., NOON, W.A., SHERMAN, P.W. and AQUADRO, C.F. 1989. DNA "fingerprinting" reveals genetic

homogeneity in colonies of the eusocial naked mole-rat. Nature (submitted).

REEVE, H.K. and SHERMAN, P.W. 1989. Queen aggression in naked mole-rats: Effects of relatedness and body size. In: The Biology of the Naked Mole-Rat (Sherman et al. eds.) Princeton University Press (submitted).

LACEY, E.A. and SHERMAN, P.W. 1989. Social organization of naked mole-rat colonies: Evidence for divisions of labor. In: The Biology of the Naked Mole-Rat. (Sherman et al. eds.) Princeton University Press (submitted).

HONEYCUTT, R.L., NELSON, K., SCHLITTER, D.A., and SHERMAN, P.W. 1989. Genetic variation within and among colonies of the naked mole-rat: Evidence from nuclear and mitochondrial genomes. Princeton Univ. Press (submitted).

14.001 CRIS0131898
GENETIC IMPROVEMENT OF PROPIONIBACTERIA AND OTHER MICROORGANISMS IMPORTANT IN FOODS

GLATZ B A; Food Technology; Iowa State University, Ames, **IOWA** 50011.
Proj. No.: IOWO2827 Project Type: HATCH
Agency ID: CSRS Period: 01 JUL 87 to 30 JUN 92

Objectives: To improve strains of Propionibacterium important in the dairy industry and in propionic acid production. To understand the genetic organization of this and of other Gram-positive organisms. To develop means of gene transfer among these organisms and other Gram-positive organisms. To identify and characterize important genetic determinants in these organisms.

Approach: Plasmids native to propionibacteria will be isolated and characterized. Genes carried on plasmids will be sought, and characterized when found. Conjugations using conjugative plasmids from other Gram-positive organisms or from the propionibacteria will be established. Transformation of DNA into protoplasts or whole cells will be performed. Bacteriophage specific for propionibacteria will be sought. Mutants altered in important traits will be constructed.

Progress: 88/01 to 88/12. The goal of the research is to investigate the genetics of the propionibacteria, which are important industrial organisms. Screening of the culture collection of 119 strains of propionibacteria for the presence of plasmid DNA has been completed. Twenty strains were found to contain plasmids, and at least 10 unique plasmids were identified in these strains. Seven plasmids were partially characterized by restriction endonuclease analysis, and restriction maps were constructed for four of these. Hybridization studies were conducted to determine the relationships among the seven plasmids that were partially characterized. Five of these plasmids were cured from their respective strains by chemical treatment, and all plasmidcarrying strains and cured derivatives were checked for antibiotic resistances, carbohydrate fermentations, and bacteriocine production. Three plasmid-associated traits have been identified: lactose fermentation and a possible cell-surface component on one plasmid, and cell clumping on another plasmid. The culture collection has been screened for the presence of temperate and/or inducible bacteriophage and bacteriocin production, and samples of rumen fluid, Swiss cheese whey, silage, and lake water have been tested for the presence of lytic phage or other inhibitory components. One strain, recently isolated from Swiss cheese, appears to contain a defective bacteriophage.

Publications: 88/01 to 88/12
GLATZ, B.A. and ANDERSON, K.I. (1988). Isolation and characterization of mutants of Propionibacterium strains. J. Dairy Sci. 71: 1769-1776.

14.002 CRIS0004941
CONSULTATION AND RESEARCH IN MATHEMATICAL AND STATISTICAL GENETICS

POLLAK E; Statistics; Iowa State University, Ames, **IOWA** 50011.
Proj. No.: IOWO1448 Project Type: STATE
Agency ID: SAES Period: 01 JUL 59 to 01 JAN 99

Objectives: Study of population genetics, with particular reference to balanced polymorphisms maintained by natural selection occurring in human and other species. Consultation on mathematical problems arising from workers in genetics.

Approach: Procedure will consist partly of the examination of theoretical models and will be partly in cooperation with individuals who have collected or are collecting data on genetic populations.

Progress: 88/01 to 88/12. E. Pollak provided assistance to Dr. A. R. Hallauer of the Department of Agronomy, who asked a question concerning covariances between relatives when a population originally has a Hardy-Weinberg structure and successive generations are produced by self fertilization. Let FS(subscript n) be the mean of a full sib family resulting from a cross between two plants that are produced after n generations of selfing. It was verified that the covariance between FS(subscript 0) and FS(subscript 4) is equal to the covariance between full sib offspring of individuals of generation 0. Assistance was also provided to Mr. Brad Hedges, a student in the Department of Agronomy. He was faced with the problem of calculating what family size is large enough so that, if there are two possible sets of underlying frequencies of K types of offspring of a cross, the probabilities of the two kinds of misclassification are each 0.025. Professor C. P. Cox of the Department of Statistics and E. Pollak collaborated in solving this problem. Previously, the solution was known only if there are two types of offspring.

Publications: 88/01 to 88/12
JUNG, Y. C., ROTHSCHILD, M. F., FLANAGAN, M. P., POLLAK, E. and WARNER, C. M. Genetic variability between two breeds based on restriction fragment length polymorphisms (RFLPs) of major histocompatability complex class I genes in the pig.

15.001 CRIS0133059
FLOW CYTOMETRIC DETERMINATION OF INSECT DNA FOR IDENTIFICATION AND CONTROL OF INSECTS

JOHNSON J S; Entomology; Texas A&M University, College Station, **TEXAS** 77843.
Proj. No.: TEXO6916 Project Type: HATCH
Agency ID: CSRS Period: 18 SEP 87 to 31 AUG 92

Objectives: 1) Develop flow cytometry and in particular total nuclear DNA content determination as a cytotaxonomic tool, for the recognition of host races, biotypes, and crytic species of insects. 2) Catalogue the nuclear DNA content of insects of economic interest. 3) Develop multiparameter DNA flow cytometry using dyes which are specific for total DNA, adenine and thymine rich repetitive DNA, and guanine and cytosine rich repetitive DNA. 4) Demonstrate the developmental pattern and mode of inheritance of DNA content variation for both euchromatic (unique) and heterochromatic (repetitive) insect DNA sequences. 5) Develop methodology to sort insect nuclei and chromosomes as an aid to gene cloning in insects.

Approach: Assay the total DNA, and sequence specific DNA content within and between individuals, populations, races, and species of economically important insects as an aid to monitoring insects of economic interest which are subjects of IPM control efforts.

Progress: 88/01 to 88/12. We have now had one year of work with this unique new methodology for the study of heritable variation in insects. We have measured the DNA content of over 1500 individual fire ants including 3 native and two imported species. In addition, we have measured the DNA content of over 200 honey bees, nearly 300 boll weevils, plus smaller number of individuals from 10 other species. The results have been unexpected and exciting. In particular we find: DNA content is a diagnostic tool that aids in identification of species, populations and types. To date, very significant and predictable DNA content differences have been found between 5 fire ants species, between 4 species of boll weevil, and between the Africanized form of the honey bee, the European honey bee and the F-1 hybrid between the two forms. DNA content variation exists within and among some populations. The most extreme variation we have observed occurs in a fire ant population in Walton Co., Georgia, were 129 of 515 males, females, and workers show 3N or triploid DNA amounts. The remainder are diploid or haploid as expected. The smallest significant variation occurs between populations of the boll weevil Anthonomus grandis where DNA content changes are associated with different host plants. DNA change occurs during development. Male fire ants are haploid during early development, but double their DNA before maturity. Boll weevils are largely 2N throughout their life, although in 1/4 to 3/4 of their cells may have 15 to 20 percent additional DNA.

Publications: 88/01 to 88/12

JOHNSTON, J.S., ELLISON, J.R. and VINSON, S.B. 1989. Flow cytometric determination of insect DNA as an aid to the description, identification, and control of the imported fire ant. Vinson SB McCOWN JL (eds.) Proc. Imported Fire Ant Sympo.
JOHNSTON, J.S. and ELLISON, J.R. 1989. DNA heterogeniety within and among fire-ants of the genus Solenopsis in southern United States: Evidence from flow cytometry. Submitted to Cytometry.
JOHNSTON, J.S. and ELLISON, J.R. 1988. Triploidy in a polygynous population of the imported fire ant Solenopsis invicta in Walton Co., Georgia. Submitted to Hereditas.
ELLISON, J.R., VINSON, S.B. and JOHNSTON, J.S. 1989. A method for determination of DNA content of nuclei of insects by flow cytometry. Submitted to Cytometry.
ELLISON, J.R., VINSON, S.B. and JOHNSTON, J.S. 1989. Acquired somatic diploidy in males of the imported fire ant. Submitted to Canadian Jn. Genet. and Cytogenet.

17.001* CRIS0133059
FLOW CYTOMETRIC DETERMINATION OF INSECT DNA FOR IDENTIFICATION AND CONTROL OF INSECTS

JOHNSON J S; Entomology; Texas A&M University, College Station, **TEXAS** 77843.
Proj. No.: TEX06916 Project Type: HATCH
Agency ID: CSRS Period: 18 SEP 87 to 31 AUG 92

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Approach: Assay the total DNA, and sequence specific DNA content within and between individuals, populations, races, and species of economically important insects as an aid to monitoring insects of economic interest which are subjects of IPM control efforts.

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ELLISON, J.R., VINSON, S.B. and JOHNSTON, J.S. 1989. Acquired somatic diploidy in males of the imported fire ant. Submitted to Canadian Jn. Genet. and Cytogenet.

21.001* CRIS0133059
FLOW CYTOMETRIC DETERMINATION OF INSECT DNA FOR IDENTIFICATION AND CONTROL OF INSECTS

JOHNSON J S; Entomology; Texas A&M University, College Station, **TEXAS** 77843.
Proj. No.: TEX06916 Project Type: HATCH
Agency ID: CSRS Period: 18 SEP 87 to 31 AUG 92

Objectives: 1) Develop flow cytometry and in particular total nuclear DNA content determination as a cytotaxonomic tool, for the recognition of host races, biotypes, and crytic species of insects. 2) Catalogue the nuclear DNA content of insects of economic interest. 3) Develop multiparameter DNA flow cytometry using dyes which are specific for total DNA, adenine and thymine rich repetitive DNA, and guanine and cytosine rich repetitive DNA. 4) Demonstrate the developmental pattern and mode of inheritance of DNA content variation for both euchromatic (unique) and heterochromatic (repetitive) insect DNA sequences. 5) Develop methodology to sort insect nuclei and chromosomes as an aid to gene cloning in insects.

Approach: Assay the total DNA, and sequence specific DNA content within and between individuals, populations, races, and species of economically important insects as an aid to monitoring insects of economic interest which are subjects of IPM control efforts.

Progress: 88/01 to 88/12. We have now had one year of work with this unique new methodology for the study of heritable variation in insects. We have measured the DNA content of over 1500 individual fire ants including 3 native and two imported species. In addition, we have measured the DNA content of over 200 honey bees, nearly 300 boll weevils, plus smaller number of individuals from 10 other species. The results have been unexpected and exciting. In particular we find: DNA content is a diagnostic tool that aids in identification of species, populations and types. To date, very significant and predictable DNA content differences have been found between 5 fire ants species, between 4 species of boll weevil, and between the Africanized form of the honey bee, the European honey bee and the F-1 hybrid between the two forms. DNA content variation exists within and among some populations. The most extreme variation we have observed occurs in a fire ant population in Walton Co., Georgia, were 129 of 515 males, females, and workers show 3N or triploid DNA amounts. The remainder are diploid or haploid as expected. The smallest significant variation occurs between populations of the boll weevil Anthonomus grandis where DNA content changes are associated with different host plants. DNA change occurs during development. Male fire ants are haploid during early development, but double their DNA before maturity. Boll weevils are largely 2N throughout their life, although in 1/4 to 3/4 of their cells may have 15 to 20 percent additional DNA.

Publications: 88/01 to 88/12

JOHNSTON, J.S., ELLISON, J.R. and VINSON, S.B. 1989. Flow cytometric determination of insect DNA as an aid to the description, identification, and control of the imported fire ant. Vinson SB McCOWN JL (eds.) Proc. Imported Fire Ant Sympo.
JOHNSTON, J.S. and ELLISON, J.R. 1989. DNA heterogeniety within and among fire-ants of the genus Solenopsis in southern United States: Evidence from flow cytometry. Submitted to Cytometry.
JOHNSTON, J.S. and ELLISON, J.R. 1988. Triploidy in a polygynous population of the imported fire ant Solenopsis invicta in Walton Co., Georgia. Submitted to Hereditas.
ELLISON, J.R., VINSON, S.B. and JOHNSTON, J.S. 1989. A method for determination of DNA content of nuclei of insects by flow cytometry. Submitted to Cytometry.
ELLISON, J.R., VINSON, S.B. and JOHNSTON, J.S. 1989. Acquired somatic diploidy in males of the imported fire ant. Submitted to Canadian Jn. Genet. and Cytogenet.

23.001* CRIS0004941
CONSULTATION AND RESEARCH IN MATHEMATICAL AND STATISTICAL GENETICS

POLLAK E; Statistics; Iowa State University, Ames, **IOWA** 50011.
Proj. No.: IOWO1448 Project Type: STATE
Agency ID: SAES Period: 01 JUL 59 to 01 JAN 99

Objectives: Study of population genetics, with particular reference to balanced polymorphisms maintained by natural selection occurring in human and other species. Consultation on mathematical problems arising from workers in genetics.

Approach: Procedure will consist partly of the examination of theoretical models and will be partly in cooperation with individuals who have collected or are collecting data on genetic populations.

Progress: 88/01 to 88/12. E. Pollak provided assistance to Dr. A. R. Hallauer of the Department of Agronomy, who asked a question concerning covariances between relatives when a population originally has a Hardy-Weinberg structure and successive generations are produced by self fertilization. Let FS(subscript n) be the mean of a full sib family resulting from a cross between two plants that are produced after n generations of selfing. It was verified that the covariance between FS(subscript 0) and FS(subscript 4) is equal to the covariance between full sib offspring of individuals of generation 0. Assistance was also provided to Mr. Brad Hedges, a student in the Department of Agronomy. He was faced with the problem of calculating what family size is large enough so that, if there are two possible sets of underlying frequencies of K types of offspring of a cross, the probabilities of the two kinds of misclassification are each 0.025. Professor C. P. Cox of the Department of Statistics and E. Pollak collaborated in solving this problem. Previously, the solution was known only if there are two types of offspring.

Publications: 88/01 to 88/12

JUNG, Y. C., ROTHSCHILD, M. F., FLANAGAN, M. P., POLLAK, E. and WARNER, C. M. Genetic variability between two breeds based on restriction fragment length polymorphisms (RFLPs) of major histocompatability complex class I genes in the pig.

29.001 CRIS0131991
MOLECULAR GENETIC REGULATION OF THE IMMUNE RESPONSE IN CHICKENS

GRIFFIN J A; Microbiology; University of Alabama, Birmingham, **ALABAMA** 35294.
Proj. No.: ALAR-8700064 Project Type: CRGO
Agency ID: CRGO Period: 01 AUG 87 to 31 JUL 90

Objectives: PROJ. 8700064. Analyze the DNA sequence and organization and their influence upon the timing of expression of the heavy chain variable region genes of chickens. Analyze the sequence, organization and kinetics of expression of the genes for the heavy chains of chicken IgG and IgA. Analyze the sequences involved in gene segment rearrangement and the influence of their organization and orientation upon recombination.

Approach: We have cloned from cDNA libraries made from plasma cell mRNA and are sequencing the variable and constant region genes. We will use pulse field electrophoresis of genomic DNA to map the organization of those genes in the germline. We will perform in situ hybridization of variable and constant probes with tissue sections of bursas from chickens of different developmental stages to define the timing and location of appearance of specific mRNA. Finally, we will construct various permutations of the conserved recombination recognition sequences and test them for their ability to participate in the gene segment rearrangements.

Progress: 87/08 to 88/07. During the last 12 months we have concentrated our efforts on three aspects of this project. First, we have cloned a 2.8 kilobase cDNA sequence that contains, by several preliminary criteria, the gene encoding the heavy chain of chicken IgA. This cDNA was isolated from a library made from the mRNA of a population of cells enriched for terminally differentiated plasma cells. We are sequencing this clone to confirm the identification. We have also isolated several genomic sequences that are rearranged from their germline position in plasma cells. One of these is the code for a variable region (V(H)) gene segment that is somewhat homologous with the murine T15 V(H) gene segment. Another of these rearranged sequences is homologous with a probe for the chicken constant region gene encoding the heavy chain of IgM (C(u)). This sequence is especially interesting, because it is amplified in the plasma cell DNA from some chickens, some humans, and specifically immunized mice. Finally, we have prepared a cDNA library of the V(H) codes represented in the plasma cell population in the gland of Harder by priming cDNA synthesis with a sequence within the 5′ end of the codes for IgM and IgG. We are currently sequencing all of these clones to confirm their identity and to compare with similar sequences found in B lymphocytes of earlier developmental stages.

Publications: 87/08 to 88/07
NO PUBLICATIONS REPORTED THIS PERIOD.

29.002 CRIS0090558
AVIAN SCIENCES CYTOGENETIC STUDIES

ABBOTT U K; Avian Science; University of California, Davis, **CALIFORNIA** 95616.
Proj. No.: CA-D*-AVS-4358-H Project Type: HATCH
Agency ID: CSRS Period: 12 JUL 83 to 30 SEP 88

Objectives: Produce experimentally, translocations, duplications, deletions and other chromosomal abnormalities useful in linkage and mapping studies; Conduct studies of the karyotype of mutant and specialty lines, including semen from aged birds and then subjected to freezing and storage; Examine avian cell lines for karyotype abnormalities in vitro.

Approach: Chromosomal abnormalities will be produced by x-irradiation, Cobalt 60 treatment, colcemid and other mutagenic treatments. Fertile zygotes dying at very early stages of development, i.e. during primitive streak formation will be screened for embryonic abnormalities. These are especially prominent in the progeny of aged birds and occasional females. Cultured cell lines will be processed for chromosomal abnormalities. Also semen that has frozen and stored from mutant and normal lines will be studied karyotypically.

Progress: 87/01 to 87/12. To date semen from three separate lines and from various genetic combinations has been frozen. The number of separate genes involved is 12. On the average we have samples from 4 males per line or genetic combination. Samples per male range from 1 - 12. At present representative semen samples are being tested for frozen-thawed fertility concentrating on those males with larger numbers of samples. Any line with poor sample test results will be replaced. Additional genotypes are being frozen. We hope to increase the number of genes included to approximately 20, and increase the number of high quality samples to about 10 - 12 per male.

Publications: 87/01 to 87/12
NO PUBLICATIONS REPORTED THIS PERIOD.

29.003 CRIS0089371
ADVANCED TECHNOLOGIES FOR THE GENETIC IMPROVEMENT OF POULTRY

ABPLANALP H; Avian Science; University of California, Davis, **CALIFORNIA** 95616.
Proj. No.: CA-D*-AVS-4294-RRProject Type: HATCH
Agency ID: CSRS Period: 01 OCT 87 to 30 SEP 92

Objectives: Identify, characterize, and map poultry genes by molecular and classical methods. Develop more efficient selection methods, including the utilization of genetic variation introduced by new technologies, for improving the economic traits of poultry.

Approach: Breed an inbred line free of endogenous viruses based on line UCD-054 and UCD-082 each of which has been shown to carry only one endogenous virus but differing from one another, so that recombination can yield virus-free birds. Establish a sperm bank for

current inbred lines to be used for an assessment of genetic change under artificial selection.

Progress: 88/01 to 88/12. Crosses and backcrosses between an inbred leghorn line (054) and a line of Australorp layers (Line 100) have been made. We are in the process of identifying endogenous viruses carried by line 100 in the 54(54x100) backcrosses to establish families differing by a single endogenous virus out of four expected. Inbred lines carrying the muscular dystrophy gene (mama) homozygously have been established on a defined genetic background of inbred line 003. We have identified Line 003, our main inbred background line for congenic to be susceptible to fatty liver ruptures when raised in the early winter months, but not when hatched in early summer. Further studies of this important feature are in progress.

Publications: 88/01 to 88/12

ABPLANALP, H. (1988). Selection response in inbred lines of White Leghorn chickens. Chapter 32:360-379 Proc. 2nd Int. Conf. quant. Genetics. Raleigh, N.C.

ABPLANALP, H. (1988). Poultry Genetics. INFAP/UCD Conf. Guadalajara, Mexico. 7p.

WILSON, B.W., ABPLANALP, H., BU R .R. ENTRIKIN R K. HOOPER, M.J. and NIEBERG, P.S. (1988). Inbred crosses and inherited muscular dystrophy of the chicken. Poultry Sci. 67:367-374.

ABPLANALP, H. AND NAPOLITANO, D. (1987). Genetic predisposition for fatty liver rugtures in White Leghorn hens of a highly inbred line. Poultry Sci. 66(1):52. (Abstr).

KLASING, K.C., SCONBERG, S., CALVERT, C.C. and ABPLANALP, H. (1988). Influence of pyruvic acid on the development and regression of fatty liver hemorrhagic syndrome. Poultry Sci. 67(1):104. (Abstr).

29.004 0132624

SPECIES AND STRAIN-SPECIFIC RECOMBINANT DNA PROBES FOR MYCOPLASMA GALLISEPTICUM

YAMAMOTO R; Epidemiology & Prevent Med; University of California, Davis, **CALIFORNIA** 95616.

Proj. No.: CA-V*-EPM-4870-SG
Project Type: SPECIAL GRANT
Agency ID: CSRS Period: 01 JUL 87 to 30 JUN 89

Objectives: To develop species and strain-specific recombinant DNA probes for Mycoplasma gallisepticum.

Approach: A genomic library for M. gallisepticum strains S6 and F DNA's will be constructed in E. coli strain JM83 using the plasmid UC8. Species and strain-specific DNA fragments from the plasmid will be identified by Southern and dot blot hybridization. The probes will be in DNA:DNA dot blot assays to identify mycoplasma in tissue and exudate of infected chickens and turkeys.

Progress: 88/01 to 88/12. M. gallisepticum (MG) species-specific and vaccine F-strain-specific DNA probes were used to detect MG in clinical specimens which can reduce the diagnostic turn-around time from 10-14 days to 2 days. While it was estimated that the isotopically labelled probe may detect from .01 to 1 ng of sample DNA (10(superscript 3) to 10(superscript 6) organisms), we question whether numbers of this size are always present in clinical specimens, particularly in chronic cases. Therefore, in order to enhance sensitivity we plan to use the polymerase chain reaction to amplify MG DNA in clinical specimens. Studies are in progress in labelling the probes with nonisotopic Chemiprobe system (FMC Bioproducts), but we have had problems with specificity. A study was conducted with Dr. S.H. Kleven (University of Georgia) to determine if the vaccine F-strain can replace wild-type MG in commercial layers. MG isolated from layer farms that had been vaccinated with the F-strain for at least 2 years were identified as the vaccine strain by SDS-PAGE, restriction enzyme analysis and dot-blot DNA hybridization using the F-strain specific DNA probe. The results suggest that the vaccine F-strain of MG can replace wild-type MG as hypothesized by Kleven.

Publications: 88/01 to 88/12

KHAN, M.I., KIRKPATRICK, B.C. and YAMAMOTO, R. (1987). A Mycoplasma gallisepticum strain-specific DNA probe. Avian Diseases 31:907-909.

KHAN, M.I., KIRKPATRICK, B.C. and YAMAMOTO, R. (1988). Mycoplasma gallisepticum strain-specific recombinant DNA probe. Proceedings of the XVIII World's Poultry Congress, Nagoya, Japan, pp. 1219-1221.

KHAN, M.I. and YAMAMOTO, R. (1989). Differentiation of the vaccine F-strain from other strains of Mycoplasma gallisepticum by restriction endonuclease analysis. Veterinary Microbiology. In Press.

KHAN, M.I., KIRKPATRICK, B.C. and YAMAMOTO, R. (1989). Mycoplasma gallisepticum species and strain Specific recombinant DNA probe. Avian Pathology. In press.

29.005 CRISO130041

MOLECULAR CLONING OF A GENE ENCODING AN OUTER MEMBRANE PROTEIN OF PASTEURELLA

HIRSH D C; Vet Microbiology & Immunology; University of California (vet-med), Davis, **CALIFORNIA** 95616.

Proj. No.: CA-V*-VMB-4764-CG Project Type: CRGO
Agency ID: CRGO Period: 15 AUG 86 to 14 AUG 88

Objectives: To demonstrate that an 80,000 molecular weight outer membrane protein on Pasteurella multocida of avian origin is a virulence determinant. To determine the conditions under which it is expressed. To determine its amino acid sequence.

Approach: The approach will be to clone the gene encoding the putative virulence determinant, mutate the gene and re-insert into a virulent strain of P. multocida in such a way

as to promote homologous recombination. Mutants will be tested for virulence. The cloned gene will be fused with lac Z gene. Condition of expression will be determined by measuring the production of galactosidase. The clone gene will be sequenced, and the amino acid sequence of the protein encoded determined.

Progress: 87/01 to 87/12. An iron regulated outer membrane protein (OMP) has been identified in Pasteurella multocida strain P1059. Antibodies to this protein are specific to epitopes on an iron regulated OMP of all but 1 of the 15 somatic serotypes of P. multocida. This antibody will be used to probe a cosmid library of P. multocida DNA in order to localize the gene encoding this protein. Turkeys passively immunized with antibodies specific for this protein are protected from lethal challenge with strain P1059.

Publications: 87/01 to 87/12

SNIPES, K.P., MARTIN, L.M. and HIRSH, D.C. Plasma and iron regulated expression of high molecular weight outer membrane proteins by Pasteurella multocida. Am. J. Vet. Res., In Press.

29.006 CRIS0096967
MOLECULAR CLONING OF AVIAN LEUKOSIS VIRUSES

SMITH R E; College of Vet Medicine; Colorado State University, Fort Collins, **COLORADO** 80523.
Proj. No.: COLV2055 Project Type: CRGO
Agency ID: CRGO Period: 15 SEP 85 to 31 MAR 88

Objectives: Proj 8502438. An avian leukosis virus, RAV-7, will be molecularly cloned using standard techniques. A detailed restriction map will be prepared, and a probe will be constructed which utilizes the LTR region of the genome.

Approach: The number of integrated and unintegrated genome copies will be estimated by Southern blot analysis. The process of constructing molecular recombinants between RAV-7 and RAV-1 will be started.

Progress: 88/01 to 88/12. Molecular cloning of the avian retrovirus RAV-7 was the goal of this project, but the objective was only partially accomplished. The restriction enzyme. SacI cut the RAV-7 genome in a single site, allowing. ligation to lambda gtWES bacteriophage aims. Bacteriophage were packaged, and plated on E. coli BNN45, followed by hybridization to a RAV-2 probe. Ten positive plaques were identified, and plaque purified-3 times. Two of these plaques were further characterized, using appropriate restriction enzymes, to prepare them for plasmid insertion. Genome-sized. DNA (7.5 kb) was ligated into the plasmid pUC12, but no stable integrations were obtained. Furthermore, no successful transformation of chicken embryo fibroblasts were obtained. We were successful in completing a comprehensive biological characterization of RAV-7. The virus causes a profound immunosuppression in infected chickens, and a generalized lymphoproliferative disease. In addition, the virus causes a neurological disease that appears to involve a severe compromise of the central nervous system. The pathological characteristics of the RAV-7-induced CNS disease were described as a nonsupporative meningoencephalitis. The inflammatory infiltrate consisted of lymphocytes, macrophages, and plasma cells. The physiological measurements conducted in our studies indicated that the nerve conduction velocities for RAV-7-infected spinal cords was half that of uninfected chickens.

Publications: 88/01 to 88/12

HEIDRICH, J.E., M.A. ADCOCK, C. BOLIN, N.F. CHEVILLE, and R.E. SMITH. Effect of Rouse associated virus number 7 on lymphoid cells and tissues of the chicken. Vet. Immunol. Immunopath. 15:267-283, (1987).
WHALEN, L.R., D.W. WHEELER, D.H. GOULD, S.A. FISCUS, L.C. BOGGIE, and R.E. SMITH. Functional and structural alterations of the nervous system induced by avian retrovirus RAV-7. Microbial Pathogenesis 4:401-416, (1988).

29.007 CRIS0138262
ADVANCED TECHNOLOGIES FOR THE GENETIC IMPROVEMENT OF POULTRY

CLARK S H; SOMES R JR; PIERRO L J; Animal Genetics Research Unit; University of Connecticut, Storrs, **CONNECTICUT** 06268.
Proj. No.: CONS00632 Project Type: HATCH
Agency ID: CSRS Period: 01 JUL 89 to 30 SEP 92

Objectives: Identify, characterize, and map poultry genes by molecular and classical methods.

Approach: Experiments will establish genetic linkage or lack thereof for an array of mutations that alter skeletal formation or pattern formation and a number of cloned "candidate" genes known to be associated with skeletal development or thought to affect pattern formation. Mutations currently being examined include Cp, ch, mm-H, mm-A, Po, dp-3, dp-5 and wg-2. The cloned "candidate" genes include collagen type II, collagen type I, collagen type IX, collagen X, collagen type XII, fibronectin and core protein. In addition, cloned genes will be isolated by screening chicken genomic and cDNA libraries with selected cloned homeotic (Drosophila) genes and linkage tested in classical breeding experiments combined with an assessment of cloned gene linkage utilizing DNA polymorphisms as molecular markers. DNA polymorphic sites will be determined utilizing denaturing gradient gel electrophoresis.

29.008 CRIS0007144
INHERITANCE & GENE ACTION OF POULTRY MUTATIONS

SOMES R G JR; Nutritional Sciences; University of Connecticut, Storrs, **CONNECTICUT** 06268.
Proj. No.: CONS00346 Project Type: STATE
Agency ID: SAES Period: 01 OCT 85 to 30 SEP 90

Objectives: To study the inherited basis of poultry and avian mutations; 2) to establish linkage relationship between these genes and those controlling other characteristics; 3) to study the physiologic and/or morphologic basis of the gene action involved; and 4) to establish if these mutant genes are associated with genes controlling economic traits.

Approach: The necessary crosses will be made to determine the inheritance and to establish the linkage group relationships and crossover unit distances for each mutant gene studied. Studies will be designed to determine the physiologic and/or morphologic basis of these mutants, using the methods most appropriate for the type of mutant under study. Where applicable, populations will be compared for a number of performance measurements to determine if linked and/or pleiotropic associations exist between a particular mutant gene and traits of economic importance.

Progress: 86/10 to 87/09. A number of ongoing studies dealing with avian mutant traits, preservation of poultry stocks, linkage and gene recording are covered by this project. Work continues on studies dealing with the inheritance of plumage color mutants in the Guinea Fowl and peafowl. Also cooperative work continues with the SUNY Downstate Medical Center on the inheritance of polymorphisms in both the light and heavy myoglobin chains of the domestic fowl and with the Southwestern Medical School at Dallas, Texas on the henny feathering gene in the domestic fowl. The new sixth edition of the Registry of Poultry Genetic Stocks has been worked on for most of the year and this edition is now almost ready for press.

Publications: 86/10 to 87/09
SOMES, R.G., JR. 1987. Linked loci of the chicken. Gallus gallus (G. domesticus). Genetic Maps 4:422-429.

29.009 CRIS0136202
LARYNGOTRACHEITIS VIRUS: IDENTIFICATION AND CHARACTERIZATION OF GLYCOPROTEINS

KEELER C L; Animal Science & Agri Biochem; University of Delaware, Newark, **DELAWARE** 19711.
Proj. No.: DEL00309 Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 88 to 30 SEP 93

Objectives: Produce immunological reagents to laryngotracheitis virus, hyperimmune sera and monoclonal antibodies against structural proteins. Create a genomic library and restriction map of laryngotracheitis virus DNA. Identify and clone laryngotracheitis virus glycoprotein genes.

Approach: A laryngotracheitis virus genomic library and preliminary restriction map will be created using standard recombinant DNA techniques. Similarly, standard procedures will be used to generate panels of monoclonal antibodies against laryngotracheitis virus proteins, virions or nucleocapsids. Laryngotracheitis glycoproteins will be identified via immunoprecipitations and PAGE analysis. Glycoprotein genes will be identified and cloned using heterologous DNA probes and monospecific antibodies.

29.010 CRIS0136831
IDENTIFICATION AND CHARACTERIZATION OF GENES OF MAREK'S DISEASE VIRUS

MORGAN R W; ANDERSON A S; Animal Science & Agri Biochem; University of Delaware, Newark, **DELAWARE** 19711.
Proj. No.: DEL07912 Project Type: STATE
Agency ID: SAES Period: 01 JUN 88 to 31 DEC 88

Objectives: The objectives of the research project are 1) to identify genes and gene products of Marek's disease virus involved in eliciting immune responses against the virus, and 2) to identify regulatory genes and gene products controlling replication and latency.

Approach: Two major approaches are used to meet objectives 1 and 2. Expression vector cloning in the bacteriophage vector lgt11 is used to identify genes whose products elicit antibody responses. Transfection of Marek's disease virus DNA into chicken embryo fibroblasts and lymphoblastoid cells is being used to identify regulatory genes.

Progress: 88/01 to 88/12. The bacteriophage expression vector lgt11 has been used to identify genes whose products elicit antibody responses against Marek's disease virus (MDV) in chickens. Two libraries have been made and screened with a variety of antibody reagents including antisera prepared against denatured MDV proteins and convalescent antisera from MDV-infected chickens. Using this approach, two open reading frames were identified that encode products bound by antibody. These open reading frames have been mapped to the MDV genome and are currently being sequenced. Future plans include continuing the characterization of these open reading frames. Co-transfection of total DNA from MDV-infected chicken embryo fibroblasts with purified BamHI clones of the viral genome has been used to identify regions of the viral genome that have a negative effect on plaque formation following transfection. Active regions, which map to the inverted and terminal repeat regions of the viral genome, will be further analyzed in future experiments.

Publications: 88/01 to 88/12
MORGAN, R.W., MCDERMOTT, C.H. and CANTELLO, J.L. 1988. Regions of the Marek's disease virus genome that inhibit transfection of MDV DNA into chicken embryo fibroblasts. Proc. of the Third Intnl. Symp. on Marek's Disease, Japan.

MORGAN, R.W., MCDERMOTT, C.H., and CANTELLO, J.L. 1988. Transfection of chicken embryo fibroblasts with Marek's disease virus DNA. Abstracts of the 88th Annual Meeting of the American Society for Microbiology, S15, p. 317.

29.011 CRIS0136409
SEX-SPECIFIC DNA IN LIVESTOCK ANIMALS

MCGRAW R A; College of Vet Medicine; University of Georgia, Athens, **GEORGIA** 30602.
Proj. No.: GEOV-0186 Project Type: STATE
Agency ID: CSVM Period: 01 JUL 87 to 30 JUN 91

Objectives: This project is aimed at identifying and characterizing sex-specific DNAs in economically important livestock species. The basic genetic information can be used to develop sex-specific DNA probes with potential application in assays for sex-fractionation of semen and/or sex determination of embryos.

Approach: The approach is to compare DNAs derived from male and female animals of each species by a variety of molecular genetic methods, including restriction analysis, cloning, sequencing, and hybridization techniques. DNA sequences unique to one of the sexes are then characterized and developed as sex-specific hybridization probes.

Progress: 87/07 to 88/12. This research is aimed at identifying and characterizing sex-specific DNAs in economically important livestock species. The genetic information is used to develop sex-specific DNA probes with potential application in assays for sex-fractionation of semen and in sex-identification of embryos. Methods include a variety of DNA manipulations: restriction enzyme digestions, electrophoretic separations, construction and propagation of recombinant DNA in bacteria, DNA sequece analysis, chemical DNA synthesis, enzymatic DNA amplification, and hybridizations using radioactively labelled probes. At this time, we have developed sex-specific probes in pigs and chickens. The procine probe has been used successfully for sex-identification of procine embryos and efforts are underway to attempt sex-fractionation of boar semen. Preliminary data suggests that we will be able to develop similar probes in horses and cattle.

Publications: 87/07 to 88/12

MCGRAW, R.A., JACOBSON, R.J. and AKAMATSU, M. 1988. A male-specific repeated DNA sequence in the domestic pig. Nucleic Acids Research 16(21):10389.

29.012 CRIS0096792
FOWLPOX VIRUS AS A CLONING VECTOR FOR POULTRY PATHOGENS

TRIPATHY D N; YUEN-WONG P H; WONG P K Y; Veterinary Research; 1301 West Gregory Drive, Urbana, **ILLINOIS** 61801.
Proj. No.: ILLU-70-0501 Project Type: CRGO
Agency ID: CRGO Period: 01 SEP 85 to 31 AUG 89

Objectives: Proj 8502178. Restriction endonuclease analysis of fowlpox virus strains. Development of a recombinant vector for insertion of foreign DNA into fowlpox virus.

Approach: DNA isolated from fowlpox virus strains will be subjected to restriction endonuclease analysis. Restriction endonuclease fragments of fowlpox virus will be cloned into plasmids. Identification of the DNA fragment containing thymidine kinase (TK) gene will be done by marker rescue. After mapping of the TK regulatory sequence, construction of a fowlpox virus cloning and expression vector will be achieved.

Progress: 87/10 to 88/09. Studies on development of fowlpox virus as an expression vector were continued. Since the foreign gene, chloramphenicol acetyltransferase (CAT), was transiently expressed under the control of a vaccinia virus promoter in cells infected with fowlpox virus, the ability of two vaccinia virus promoters to control expression of foreign genes in fowlpox virus recombinants was examined. For this purpose, plasmid vectors capable of directing the insertion of a vaccinia virus P7.5 of P11 promoter-Escherichia coli B-galactosidase (B-gal) gene fusion into the thymidine kinase gene of fowlpox virus were constructed. Following plasmid transfection of fowl poxvirus-infected cells, the progeny were visually screened for recombinant viruses which produced blue plaques in the presence of the B-gal indicator, X-gal. The predicted location of the B-gal gene in one such recombinant was verified by hybridization analysis. The constitutive and late regulatory natures of the vaccinia virus P7.5 and P11 promoters respectively, were conserved. Moreover, a slightly higher level of expression was obtained when using the P-11 promoter as compared to the P7.5 one.

Publications: 87/10 to 88/09

SCHNITZLEIN, W.M., GHILDYAL, N., and TRIPATHY, D.N. (1988). Genomic and antigenic characterization of avipoxviruses. Virus. Res. 10:65-76.
SCHNITZLEIN, W.M., GHILDYAL, N., and TRIPATHY, D.N. (1988). A rapid method for identifying the thymidine kinase genes of avipoxviruses. J. Virol. Methods 20:341-352.
SCHNITZLEIN, W.M., and TRIPATHY, D.N. (1988). Recognition of a vaccinia virus promoter by a recombinant fowlpox virus, Abst. and Poster presentation. "Vector-Based Vaccines." The 1988 Albany Conferences, Albany, NY.
SCHNITZLEIN, W.M., and TRIPATHY, D.N. (1988). Utilization of vaccinia virus promoters by recombinant fowlpox viruses. Abst. 69th Ann. Mtg. Conf. Res. Workers Anim. Dis., Chicago.

29.013 CRIS0088111
ADVANCED TECHNOLOGIES FOR THE GENETIC IMPROVEMENT OF POULTRY

LAMONT S J; WARNER C M; Animal Science; Iowa State University, Ames, **IOWA** 50011.
Proj. No.: IOW02576 Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 87 to 30 SEP 92

Objectives: Identify, characterize, and map poultry genes by molecular and classical methods. Develop and test procedures to apply emerging technologies to poultry breeding by theoretical modeling, simulation, and animal testing.

Approach: Genes of the chicken major histocompatibility complex (MHC) will be isolated by screening genomic and cDNA libraries from inbred chickens with homologous and heterologous MHC probes. Cloned genes will be analysed to determine DNA sequence. The chicken MHC genes will be mapped by restriction fragment length polymorphism (RFLP) analysis, and by chromosome walking. Selected chicken lines will be established, characterized, and maintained for study of the molecular structure of the MHC. The feasibility of using RFLP analysis in poultry breeding programs will be examined by determining correlations of RFLP patterns with traits of economic importance.

Progress: 88/01 to 88/12. This project focuses on molecular genetic analysis of the chicken major histocompatibility complex. We constructed a library of genes from an inbred chicken and have isolated three chicken MHC gene clones. Mapping the genes showed that each of the three clones was unique. The DNA sequence of the subclone containing the MHC gene from two of these three clones was determined. The orientation of the coding regions was determined. Comparison of the DNA sequences showed that the chicken MHC genes sequenced here are evolutionarily related to similar MHC genes of chickens of different MHC types and the MHC genes of other species. This is the first reported cloning of chicken class II MHC genes in the U.S. Knowledge of the molecular genetic structure of the chicken MHC will lead to a better understanding of the differences between MHC types that determine resistance to disease. Direct study of MHC differences at the DNA level were conducted by using RFLP analysis. Sperm DNA was isolated from chickens of the ISU S1 line, which have been selectively bred for MHC type, immune response, and disease response traits. Difference in RFLP patterns were found to be associated with MHC type. This shows that RFLP analysis may be a useful addition to MHC studies in the chicken. Red blood cell DNA was isolated from chickens from the USDA Regional Poultry Laboratory.

Publications: 88/01 to 88/12

WARNER, C. M., M. F. ROTHSCHILD, and S. J. LAMONT (eds). (1988). The Molecular Biology of the Major Histocompatibility Complex of Domestic Animal Species. Iowa State University Press, Ames, Iowa.

LAMONT, S. J. Biotechnology in poultry breeding. Egg Industry 94:22-23, (1988).

LAMONT, S. J. Immunogenetics, the major histocompatibility complex, and disease resistance in poultry. Pp 185-192. In: Proc. 38th Western Poultry Disease Conference. Davis, CA.

PITCOVSKI, J., S. J. LAMONT, A. W. NORDSKOG, and C. M. WARNER, (1989). Analysis of B-G and immune response genes in the Iowa State University S1 chicken line hybridization of sperm DNA with a major histocompatibility complex class II probe.

LAMONT, S. J. (1989). The chicken major histocompatibility complex in disease resistance and poultry breeding. J. Dairy Sci. (in press).

XU, Y., PITCOVSKI, J., PETERSON, L., AUFFRAY, C., BOURLET, Y., GERNDT, B., NORDSKOG, A. W. LAMONT, S. J. and WARNER, C. W. (1989). Isolation and characterization of three class II major histocompatibility complex genomic clones from the ch.

29.014 CRIS0096981
MOLECULAR ANALYSIS OF THE CHICKEN MAJOR HISTOCOMPATIBILITY COMPLEX

WARNER C M; LAMONT S J; Biochemistry; Iowa State University, Ames, **IOWA** 50011.
Proj. No.: IOW-8502847 Project Type: CRGO
Agency ID: CRGO Period: 01 SEP 85 to 30 SEP 88

Objectives: Proj. 8502847. Prepare genomic and cDNA libraries from two chicken lines congenic at the B complex, BHs-B and GHs-B . Screen the libraries with mouse and human DNA probes for Class I, Class II, and Class III MHC genes. Isolate and clone the genes detected with the probes. Do restriction mapping on the isolated clones to determine the number of unique clones isolated. Determine the DNA sequence of each unique clone. Compare the sequences of the cDNA vs. genomic DNA clones and the GHs-B vs. GHs-B clones. Determine RFLP patterns among the 14 inbred chicken lines. Start to "walk" up and down the B complex with the long range goal of determining the DNA sequence of the whole B complex.

Approach: Construct genomic libraries from 15-20 kb chicken genomic DNA and a vector. Construct cDNA libraries from poly A mRNA. Screen libraries with nick-translated mouse and human DNA Class I, II, and III probes. Amplify positive clones by infecting E. coli and isolating plasmid on phage DNA. Sequence DNA by both Maxam and Gilbert and the Sanger procedure. Use Southern blot procedure to detect the number of unique clones, and also to compare restriction fragment length polymorphisims between inbred lines.

Progress: 87/10 to 88/09. In the last year of this grant we have accomplished many of our goals of defining the genes of the chicken major histocompatibility complex (MHC). These genes are involved in the control of the immune response and disease resistance. We have completed the sequence of the DNA of two class I MHC genomic genes from a chicken of the B haplotype. We are in the process of determining the sequence of several cDNA clones from a B chicken. The long range goal is to understand

how the structure of these genes is related to function. For instance, some chickens are resistant to certain pathogens while others are not. The MHC genes will be prime candidates for the future genetic engineering of chickens.

Publications: 87/10 to 88/09

WARNER, C.M., ROTHSCHILD, M.F. and LAMONT, S.J. (eds.) 1988. Proceedings of the International Symposium on the Molecular Biology of the Major Histocompatibility Complex of Domestic Animal Species. Iowa State University Press., 202 pp.

XU, Y., PITCOVSKI, J., PETERSON, L., AUFFRAY, C., BOURLET, Y., GERNDT, B.M., NORDSKOG, A.W., LAMONT, S.J. and WARNER, C.M. 1989.

PITCOVSKI, J., LAMONT, S.J., NORDSKOG, A.W. and WARNER, C.M. 1989. Analysis of B blood group and immune response genes in the Iowa State University S1 chicken line by hybridization of sperm DNA with an MHC class II probe. Poultry Sci.

WARNER, C.M., GERNDT, B.M., XU, Y., BOURLET, Y., AUFFRAY, C., LAMONT, S.J. and NORDSKOG, A.W. 1989. Restriction fragment length polymorphism (RFLP) analysis of MHC class II genes from inbred chicken lines. Anim. Genetics, submitted.

29.015 CRIS0133928

REGULATION OF THE AMOUNT OF BODY FAT IN POULTRY

GOODRIDGE A G; Biochemistry; University of Iowa, Iowa City, **IOWA** 52242.

Proj. No.: IOWR-8800001 Project Type: CRGO
Agency ID: CRGO Period: 15 DEC 87 to 30 SEP 90

Objectives: PROJ. 8800001. Our objective is to develop methods for the selective modification of specific metabolic pathways in intact cells and animals.

Approach: As a model, we propose to alter the rate of de novo fatty acid biosynthesis in growing quail. The pace-setting enzyme for the de novo synthesis of long-chain fatty acids is acetyl-CoA carboxylase. Malic enzyme provides a large fraction of the NADPH required for fatty acid synthesis. We propose to isolate a cDNA clone for avian acetyl-CoA carboxylase and use it, and existing malic enzyme cDNA clones, to construct chimeric anti-genes which contain the promoter/enhancer sequence of Rous Sarcoma Virus and the structural sequence, in reverse orientation, of the cDNA for acetyl-CoA carboxylase or malic enzyme. The effectiveness of these chimeric anti-genes will be tested in fibroblasts in culture. If the cell culture experiments are successful, we will construct transgenic quail which contain the anti-genes. In principle, the approaches developed in this proposal also should be applicable to any avian or mammalian species.

Progress: 88/01 to 88/12. Our objective is to construct cells and animals in which the expression of the endogenous genes for malic enzyme or acetyl-CoA carboxylase has been inhibited. The long-term goal is to use genetic engineering to construct a line of chickens which with substantially lowered rates of lipogenesis. Our initial plasmid construct contained the 5'-end of malic enzyme of cDNA in reverse orientation and used the 5'LTR of Rous sarcoma virus as it promoter-enhancer. We were unable to detect expression in QT-6 cells. We are preparing new constructs with the same sequence element located in a shuttle vector which contains a selectable marker. We also are preparing retroviral vectors which contain anti-sense malic enzyme and sense human growth hormone. The virus's own promoter-enhancer (5'LTR) will drive transcription and the human growth hormone sequence will add stability to the transcripts. We have found that anti-sense oligodeoxynucleotides added to the medium of chick-embryo hepatocytes cause a small inhibition of the expression of the cognate protein. Anti-sense acetyl-Co carboxylase inhibits expression of acetyl-CoA carboxylase but not malic enzyme. Anti-sense malic enzyme inhibits expression of malic enzyme but not acetyl-CoA carboxylase. Maximum inhibition is only about 30% and requires a high concentration of oligodeoxynucleotide. We are collaborating with Dr. Joe Walder of this department on this project.

Publications: 88/01 to 88/12

NO PUBLICATIONS REPORTED THIS PERIOD.

29.016 CRIS0099974

STRUCTURE AND EXPRESSION OF CHICKEN METALLOTHIONEIN GENES

ANDREWS G; University of Kansas, Kansas City, **KANSAS** 66103.

Proj. No.: KANR-8601263 Project Type: CRGO
Agency ID: CRGO Period: 01 SEP 86 to 28 FEB 89

Objectives: Project 8601263. The objective of this proposal is to determine the structure and regulation of metailothionein genes in the chicken. The effects of glucocorticoid hormones, lipopolysaccharides and metal ions on expression of metallothionein genes will be determined in order to examine mechanisms of regulation of this gene in birds exposed to environmental stresses. Furthermore, expression of metallothionein genes during embryonic development will be examined in order to gain some insight into the functional aspects of this protein.

Approach: Metallothionein genes will be purified from lambda phage-genomic DNA libraries and the nucleotide sequence of these genes will be determined by the Maxam and Gilbert sequencing technique. Cloned metallothionein genes will be used to quantitate relative rates of gene transcription in polymerase run-off transcription assays. Levels of metallothionein messenger RNA will be determined by Northern blot hybridization analysis following exposure of birds to various inducers. Using these methods, expression of metallothionein genes will also be analyzed in embryonic tissues at various stages of development.

Progress: 87/09 to 88/08. Grant close out. Several chicken metallothionein (MT) cDNA clones were obtained, and the nucleotide sequences of longest (376 bp) of these clones

were determined. The deduced amino acid sequence of chicken MT indicates a protein of 63 amino acids of which 20 are cysteine residues. These results were confirmed by amino acid composition and partial amino acid sequence analyses of the purified chicken MT protein. In the chicken liver, levels of chicken metallothionein mRNA were rapidly induced to high levels by metals (Cd, Zn, Cu), glucocorticoids and lipopolysaccharides. Chicken metallothionein mRNA levels were modest in the embryonic liver, peaking on around day 15 of development, declining to basal levels until hatching, and rising again during the first week posthatching. Southern blot analysis of chicken DNA indicates that the chicken MT gene is likely to be a unique gene sequence. A chicken genomic DNA-lambda phage library was screened using a chicken MT cRNA probe. Seven positive phage were identified and purified. Restriction enzyme mapping, and hybridization analyses showed that each phage contained a 20 kb common stretch of chicken DNA, and a single chicken MT gene. Both strands of the gene and 5′-flanking region were sequenced and the chicken MT gene was found to be about 1.5 kb in length. A comparison of the nucleotide sequences of the gene and the cDNa revealed the presence of three exons of 96, 66, 226 bp, separated by two intervening sequences of 87 and 1010 bp.

Publications: 87/09 to 88/08

WEI, D. and ANDREWS, G.K. Molecular Cloning of Chicken Metallothionein: Deduction of the Complete Amino Acid Sequence and Analysis of Expression Using Cloned cDNA. Nucl. Acids Res., 16: 537-553, 1988.

FERNANDO, L.P., WEI, D. and ANDREWS, G.K. Structure and Expression of Chicken Metallothionein. J. Nutrit. In Press.

29.017 CRIS0049916

CLONING DNA OF COCCIDIAN AND HELMINTH PARASITES FOR STRAIN IDENTIFICATION AND VACCINE PREPARATION

DAME J B; Biostematics Laboratory Animal Parasitology Institute; Beltsville Agr Res Center, Beltsville, **MARYLAND** 20705.

Proj. No.: 1265-34000-002-00D
Project Type: INHOUSE
Agency ID: ARS Period: 01 JUL 85 to 01 JUL 90

Objectives: 1) Develop a reliable system to distinguish between related strains and/or species of Trichinella, Ostertagia, Haemonchus and Eimeria based on differ-ences in the structure of their DNA; 2) clone and express in Escherichia coli, genes from Toxoplasma gondii and Eimeria spp., which encode antigens that may be useful in preparing vaccines or diagnostic tests for these parasites.

Approach: 1) Genomic DNA libraries will be prepared in plasmids of Escherichia coli and clones of repeated DNA sequences, ribosomal RNA genes and mitochondrialDNA identified. These clones will be used as DNA hybridization probes to detect strain and species differences by southern blot analysis. 2) Geno- mic DNA libraries will be prepared in the E. coli expression vector gambda gt11 by the mung bean nuclease method. Clones of genes encoding parasite antigens will be identified using antibodies. Recombinant-produced anti- gens will be compared with native antigens and their value as a vaccine component tested.

Progress: 88/01 to 88/12. Ribosomal DNA genes were cloned from Trichinella spiralis and subcloned in plasmid vectors for use as DNA probes. These subcloned fragments were useful in studying the systematics and in the characterization of T. spiralis (pig biotype) and T. pseudospiralis. A cloned DNA sequence was also identified from a T. spiralis cDNA expression library which has application in serodiagnosis. The characterization of these gene sequences provided information on the excretory-secretory antigens of this parasite. We isolated DNA from ethanol fixed cestode proglottids and determined genetic markers that support the recognition of a new species of Taenia from Taiwan. In an attempt to find an antigen for use in a vaccine against coccidiosis of chickens, DNA encoding a recombinant antigen of Eimeria acervulina merozoites was cloned after immunoscreening bacteriophage expression libraries. The purified recombinant antigen, believed to be a series of related surface proteins, stimulated T cells from Eimeria-immune chickens and when administered to susceptible chickens conferred partial protection. Sequencing of a cDNA encoding a portion of a second E. acervulina merozoite p250 surface protein revealed tandem-repeated DNA similar to structures reported for malaria. Similarities in protein structure between different protozoans of the Apicomplexa may imply similar means of evading immune responses by the host.

Publications: 88/01 to 88/12

ZARLENGA, D.S. and DAME, J.B. 1988. Molecular cloning and characterization of ribosomal RNA genes from Trichinella spiralis. The FASETS Journal 2(5):A 1028.

JENKINS, M.C. 1988. A cDNA encoding a merozoite surface protein of the protozoan Eimeria acervulina contains tandem-repeated sequences. Nucleic Acids Research. 16(20):9863.

JENKINS, M.C., DAME, J.B., LILLEHOJ, H.S., DANFORTH, H.D. and RUFF, M.D. 1988. Cloned genes coding for avian coccidiosis antigens. U.S. Patent Application, 7-155,264.

JENKINS, M.C., LILLEHOJ, H.S. and DAME, J.B. 1988. Eimeria acervulina: DNA cloning and characterization of recombinant sporozoite and merozoite antigens. Exp. Parasitol. 66:96-107.

LILLEHOJ, H.S., JENKINS, M. C., BACON, L.D., FETTERER, R.H. and BRILES, W. E. 1988. Protection against Eimeria acervulina correlates with T cell response to r ecombinant surface merozoite antigen. Exp. Parasitol. 67:148-158.

JENKINS, M.C., LILLEHOJ, H.S., DANFORTH, H.D. and FETTERER, R.H. 1988. cDNA encoding antigens of Eimeria acervulina: DNA sequence analysis; T cell and B cell epitopes. Ann. Mtg. Fed. Exp. Biol., Las Vegas, NV (ABSTRACT).

JENKINS, M.C., STROHLEIN, D.A., DANFORTH, H.D. and LILLEHOJ, H.S. 1988. Cloning of genes encoding surface antigens of Eimeria acervulina sporozoites and merozoites. Ann. Mtg. Poult. Sci. Ass., Baton Rouge, LA (ABSTRACT).

29.018 CRIS0140695
ISOLATION AND ANALYSIS OF CHICKEN MHC CLASS II GENES AND RELATION TO DISEASE

BACON L D; GREENLEE A R; SMITH E J; Avian Leukosis Research Unit; Agricultural Research Service, East Lansing, **MICHIGAN** 48824.
Proj. No.: 0101-21020-008-40R
Project Type: GRANT
Agency ID: ARS Period: 15 SEP 85 to 30 SEP 89

Objectives: 1. To isolate bacteriophage cDNA clones that contain DNA sequences for class II antigens. 2. To use these cDNA clones as probes to identify class II MHC polymorphisms (differences) in chicken DNA of strains with standard MHC (B) haplotypes, and of other characterized strains, so that the associ-ation between disease and MHC genotype can be specifically and rapidly defined.

Approach: The gene which codes for a class II molecule will be isolated by first preparing a recombinant cDNA clone bank from chicken cells. This bank will be screened with human or mouse probe(s) specific for class II gene(s). Recombinant clones will be identified and used to study restriction frag- ment length polymorphisms (RFLP) of DNA from chickens of (1) different "standard" B-haplotype strains, (2) B-congenic lines, (3) B-recombinant strains, (4) strains selected for disease resistance, and (5) different strains with similar serological types to prove they are identical by RFLP for class II genes. The clones will be used to analyze, isolate and charac-terize the chromosomal region containing the MHC and associated disease susceptibility loci of the chicken. COMPETITIVE GRANT PROJECT.

Progress: 88/01 to 88/12. In September 1988, we renewed work on this project. Initially DQ and DR.B probes specific for the beta chain genes of human class II major histocompatibility complex (MHC) molecules were obtained, and amplified in plasmid vectors for use as probes. We then had oligonucleotide probes commercially produced, specifically for short sequences of published class II genes of BL.B2 and BL.BTM, and of class I BF genes of the chicken MHC (B-haplotype). These oligonucleotide probes were labelled with 32.P for use as probes. Chicken cDNA libraries from liver, and the RP-9 cell line, were then screened with the mixed oligo probes. Ten of the positive plaques from the liver library, and 20 of the RP9 were collected and screened with the oligo mixture. Most of these plaques remained positive through the secondary screening. They were then purified and screened against each of the oligos separately. Twelve bacteriophage clones giving positive signals with BL.B2-1 and/or BL.TM-1 have been grown up in quantity and are being prepared for CsC1 centrifugation. Subsequently, they will be characterized by partial nucleotide sequencing to establish their specificity for BL, and their potential use as probes for chicken MHC class II genes. For this purpose we have prepared genomic DNA from 10th backcross chickens of the 8 15.B congenic lines.

Publications: 88/01 to 88/12
NO PUBLICATIONS REPORTED THIS PERIOD.

29.019 CRIS0140622
MOLECULAR APPROACHES TO THE IMPROVEMENT OF ECONOMIC TRAITS IN POULTRY

CRITTENDEN L B; SALTER D W; SOLLER M; Avian Leukosis Research Unit; Agricultural Research Service, East Lansing, **MICHIGAN** 48824.
Proj. No.: 4001-21020-001-26R
Project Type: INHOUSE
Agency ID: ARS Period: 01 OCT 85 to 30 SEP 88

Objectives: Develop methods for introducing genes into the germline; utilize endogenousviral genes as genetic markers at the DNA level; develop a theoretical framework for application of these methods.

Approach: Determine the most efficient way to introduce avian leukosis viruses in thegermline, and then use retrovirus vectors developed from avian leukosis viruses to introduce foreign genes. Evaluate the stability and expression of such genes. Use the endogenous viral genes found in highly productive lines of chickens as restriction fragment length polymorphisms and evaluatetheir usefullness as marker genes to assist in selection for economic traits.

Progress: 88/01 to 88/09. Studies in the United States showed that of 23 avian leukosis virus (ALV) germ-line inserts two are defective for complete virus production. One of those expressed only the viral envelope gene and was resistant to subgroup A ALV, but lacked detectable proviral alterations. The other expressed both group-specific and envelope antigens and was moderately resistant to subgroup A ALV and had a deletion in the polymerase gene which apparently caused a defect in viral reversetranscriptase. One of the inserts has been made homozygous to produce a line uniformly resistant to subgroup A ALV. In Israel, a survey of endogenous virus (ev) genes in 5 populations of chickens from different breeds has been completed. It was shown that each population of chickens was highly polymorphic and that many of the proviruses were shared by several of the lines. It is notable that few of the proviruses were present at a high gene frequency, suggesting that ev genes originated before breed divergence and that evolutionary selection may have been against homozygotes.

Publications: 88/01 to 88/09
NO PUBLICATIONS REPORTED THIS PERIOD.

29.020 CRIS0136650
REGULATORY REGIONS OF MAREK'S DISEASE HERPES VIRUS

COUSSENS P; Animal Science; Michigan State University, East Lansing, **MICHIGAN** 48824.
Proj. No.: MICL08058 Project Type: CRGO
Agency ID: CRGO Period: 15 JUL 88 to 31 JUL 90

Objectives: PROJ. 8801606. The overall goal of our research is to understand the replication, pathogenesis, and immunobiology of the Marek's disease herpesvirus. Consistent with this goal, our current project objectives are: 1. Identify promoter regions of MDHV late genes. 2. Identify MDHV genome segments which encode any trans-acting regulatory factors. 3. Prepare genetically engineered cells which express these MDHV regulatory factors.

Approach: The promoter region of the MDHV gp57-65 gene will be located by a combination of primer extension, S1 nuclease mapping (mRNA cap site), and DNase I footprinting. This region will be fused to a reporter gene (CAT). Reporter gene expression following transfection of MDHV infected CEF cells or co-transfection with various MDHV genome segments will be used as an assay of transcriptional activity from the reporter gene-gp57-65 promoter construct. Positive segments of the genome will be further tested to localize the gene encoding the regulatory factor(s). Once localized, these genes will be used to produce cells which express the appropriate trans-acting transcriptional activators necessary of expression of MDHV late glycoprotein genes. In subsequent proposals, these cells will be used to further examine MDHV gene regulation and to express MDHV glycoproteins from their endogenous promoters.

29.021 CRIS0090515
STRUCTURE/FUNCTION RELATIONSHIPS IN CHICKEN GLOBIN AND HISTONE GENES

DODGSON J; Microbiology and Public Health; Michigan State University, East Lansing, **MICHIGAN** 48824.
Proj. No.: MICL01453 Project Type: HATCH
Agency ID: CSRS Period: 12 AUG 88 to 11 AUG 93

Objectives: To continue our structural analyses of various chicken genes including the chicken histone genes, carbonic anhydrase genes, HMG genes and chick globin genes. To continue to make specific modifications in certain of these genes by in vitro gene modification techniques. To examine the function of the genes so altered by introducing them into living eucaryotic cells through the use of DNA-mediated transformation of cells in culture, and of RNA viral vectors for both cells and whole chickens.

Approach: (Numbers correspond to above in Objectives). Through the use of standard recombinant DNA techniques, i.e., nucleic and hybridization, cDNA and genomic cloning, restriction mapping, DNA sequencing, S1 mapping to transcripts, blotting techniques. Using restriction enzyme techniques to create various deletions, linker scanning mutagenesis and site-directed mutogenesis using synthetic oligonucleotides. Genes are returned to living cells by transfection techniques (Ca(3)(PO(42)) DEAE-dextran, electroporetion) and by retroviral vectors. Stable or transient transfection may be used. Generally there are done with tissue culture cells but retroviral vectors can be used in whole chickens. Gene expression is monitored at the RNA level (S1 mapping or RNA probe mapping or northern blots) and at the protein level (SDS-PAGE gel, immunoblotting techniques, CAT assays).

Progress: 88/01 to 88/12. Studies with the chicken alpha-globin genes have revealed tow negative regulatory elements which may serve to limit expression of these genes in non-erythroid tissues. Further studies also have revealed an enhancer, or positive regulatory element upstream of the alpha A-globen gene. Research continues to determine the mechanism by which these elements contribute to normal globin gene expression. Studies on the cell-cycle regulation of chicken histone genes continue. We have shown that both 5' and 3' flanking regulatory elements contribute to cell cycle control and are quantitating their relative contributions. We have isolated nearly full length cDNA clones and complete genomic clones for the chicken HMG14 and HMG17 genes. These genes code for proteins importance in the regulation of chromatin structure and gene expression. The DNA sequences of the cDNA clones have been determined, and we have nearly completed our analysis of the sequence and organization of the genomic clones. We are beginning in vitro mutagenesis experiments with both unusual histone gene variants and with the HMG14 and 17 genes which should provide valuable information regarding the function of these chromosomal proteins in vivo.

Publications: 88/01 to 88/12

DODGSON, J.B., BROWNE,D.L. and BLACK. A.J. 1988. Chicken chromosomal protein HMG-14 and HMG-17 cDNA clones: isolation, characterization and sequence comparison. Gene 63:287-295.

29.022 CRIS0098437
ISOLATION AND ANALYSIS OF CHICKEN MHC CLASS II GENES AND RELATION TO DISEASE

BACON L D; DODGSON J B; SMITH E J; Regional Poultry Laboratory; 3606 East Mt Hope Road, East Lansing, **MICHIGAN** 48823.
Proj. No.: MICR-8502587 Project Type: CRGO
Agency ID: CRGO Period: 01 OCT 85 to 31 MAR 90

Objectives: Proj. 8502587. To isolate bacteriophage cDNA clones that contain DNA sequences for class II antigens. To use these cDNA clones as probes to identify class II MHC polymorphisms (differences) in chicken DNA of strains with standard MHC (B) haplotypes, and of other characterized strains, so that the association between disease and MHC genotype can be specifically and rapidly defined.

Approach: The gene which codes for a class II molecule will be isolated by first preparing a recombinant cDNA clone bank from chicken cells. This bank will be screened with human or mouse probe(s) specific for class II gene(s). Recombinant clones will be identified and used to study restriction fragment length polymorphisms (RFLP) of DNA from chickens of (1) different "standard" B-haplotype strains, (2) B-congenic lines, (3) B-recombinant strains, (4) strains selected for disease resistance, and (5) different strains with similar serological types to prove they are identical by RFLP for class II genes. The clones will be used to analyze, isolate and characterize the chromosomal region containing the MHC and associated disease susceptibility loci of the chicken.

Progress: 87/01 to 87/12. In December 1986, Dr. Joanne Kivela discontinued her research on this project. Her progress was described in the 1986 CRIS report, and has been documented in the report and Ph.D. Dissertation cited below. During this year we have decided to recruit a Postdoctoral Fellow to continue this research. An initial candidate ultimately withdrew an acceptance of the position, and we are again seeking applicants. New approaches are being evaluated based on information gained at a conference on the MHC in Domestic Animals held in Ames, Iowa in October, 1987.

Publications: 87/01 to 87/12

KIVELA, J., KUNG, H.J., DODGSON, J. and BACON, L.D. 1987. Avian Immunology II. pp. 199-206. "Cloning of a putative chicken MHC class II alpha chain gene" ed. by Weber, W.T. and Ewert, D.L. Alan R. Liss, Inc., New York, NY.

TILLOTSON-KIVELA, J. 1987. Part A. The search for a chicken major histocompatibility complex class II alpha gene. Ph.D. Thesis, Michigan State University, East Lansing, MI. 153 pages.

29.023 CRIS0011573
ADVANCED TECHNOLOGIES FOR THE GENETIC IMPROVEMENT OF POULTRY

GUISE K S; Animal Science; University of Minnesota, St Paul, **MINNESOTA** 55108.
Proj. No.: MIN-16-017 Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 87 to 30 SEP 92

Objectives: Identify, characterize, and map poultry genes by molecular and classical methods. Develop methods for creating new genetic variation in poultry by gene transfer and chromosome alteration.

Approach: Chicken linkage groups will be identified and genes mapped to specific chromosomes through the use of classical breeding studies combined with DNA restriction fragment length polymorphism and in situ chromosome hybridization analysis. DNA libraries will be constructed and genes of economic importance molecularly cloned. Investigation of gene expression versus physiological response will be pursued using molecular probes. Gene transfer technologies will be explored and developed in tissue culture systems followed by adaptation to embryo based systems.

Progress: 88/01 to 88/12. The molecular genetics of poultry and fish were explored in the 1988 calendar year in several areas. (1) In situ hybridization of (H) labeled DNA probes to metaphase chromosome spreads showed Beta-actin sequences on chicken chromosomes 2q and one of the 9-12 group, and BKM sequences in the horse Y chromosome. (2) A putative chicken IGF-1 clone has been isolated and is under confirmation analysis. (3) M13 hypervariable region probes have shown level of homozygosity in inbred chicken lines, and variability between lines. Probes may be of use in stock identification and isolation of quantitative traits. (4) Gene transfer in chickens using non-retroviral vectors and methods continues using Beta-galactosidase and luciferase marker genes in tissue culture and whole animals. (5) Mitochondrial DNA analysis of tullibee and lake whitefish populations showed inter and intra-lake stock relationships. (6) Gene transfer has been effected in fish (goldfish and northern pike) by microinjection using neomycin resistance and bovine growth hormone genes. Transgenic pike with bovine growth hormone genes show evidence of enhanced growth rate.

Publications: 88/01 to 88/12

ANDERSEN, L., HAGAN, S., HEINKE, C. and GUISE, K.S. 1988. Semimicro Technique for DNA isolation from nucleated erythrocytes. Focus 10, 35-36.

KENT, M.G., ELLISTON, K.O., SHROEDER, W., GUISE, K.S. and WACHTEL, S.S. 1988. Conserved repetitive DNA sequences (Bkm) in normal males and sex-reversed females of the horse detectected by in situ hybridization. Cytogenet. Cell Genet.

FEHRER, S.C., SILSBY, J.L., EL HALAWANI, M.E. and GUISE, K.S. 1988. Research Note: 5-azacytidine induced changes in plasma prolactin in turkeys. Poultry Science, in press.

YOON, S.J., HALLERMAN, E.M., GROSS, M., LIU, Z., SCHNEIDER, J.F., FARAS, A.J., HACKETT, P.B., KAPUSCINSKI, A.R. and GUISE, K.S. 1988. Transfer of the gene for neomycin resistance into goldfish, Carassius auratus.

HALLERMAN, E.M., SCHNEIDER, J.F., GROSS, M., FARAS, A.J., HACKETT, P.B., GUISE, K.S. and KAPUSCINSKI, A.R. 1988. Enzymatic dechorionation of goldfish, walleye, and northern pike eggs. Trans. Am. Fisheries Soc., accepted for publication.

YOON, S.J., LUI, Z., KAPUSCINSKI, A.R., HACKETT, P.B., FARAS, A. and GUISE, K.S. 1988. Successful gene transfer in fish. 16th U.S./Japan natural Resources Panel on Aquaculture Symposium, Oct. 19-22, 1987. Charleston, S.C., in press.

YOON, S.J., LIU, Z., KAPUSCINSKI, A.R., HACKETT, P.B., FARAS, A. and GUISE, K.S.

29.024 CRIS0098029
MOLECULAR CLONING OF CHICKEN GENES INVOLVED IN MACROPHAGE DIFFERENTIATION

MURTAUGH M P; Veterinary Pathobiology; University of Minnesota, St Paul, **MINNESOTA** 55108.
Proj. No.: MINV-63-039 Project Type: ANIMAL HEALTH
Agency ID: CSRS Period: 17 FEB 86 to 30 SEP 87

Objectives: Macrophages in bone regulate bone remodeling and calcium homeostasis. This role is especially important in chickens since an enormous calcium flux from bone is required for eggshell formation and because the extremely rapid growth of broilers places severe demands on skeletal development. The common incidence of bone lesions and abnormalities in chickens indicates that bone macrophage differentiation may be abnormal resulting in nonfunctioning cells.

Approach: To understand the relation between clinical disease and macrophage dysfunction we will elucidate the molecular basis of macrophage differentiation. C-fos is a gene that seems to be involved directly in macrophage development. Thus, we will construct a genomic DNA library from chicken liver and spleen DNA and isolate the c-fos gene from it using the c-fos gene from a mouse retrovirus as a probe. The c-fos gene structure will be analyzed by restriction mapping and sequencing. The regulatory elements of the gene will be compared to other genes regulated by vitamins A and D in order to determine how these agents interact at the level of gene expression to modulate macrophage differentiation and bone development in chickens.

29.025 CRIS0134550
ADVANCED TECHNOLOGIES FOR THE GENETIC IMPROVEMENT OF POULTRY

BRUMBAUGH J A; School of Biological Sciences; University of Nebraska, Lincoln, **NEBRASKA** 68583.
Proj. No.: NEBW-3-333 Project Type: STATE
Agency ID: OCI Period: 01 OCT 87 to 30 SEP 92

Objectives: Identify, characterize, and map poultry genes by molecular and classical methods. Develop methods for creating new genetic variation in poultry by gene transfer and chromosome alteration.

Approach: Avian leukosis virus is being used as an insertional mutagen to tag 4 pigment genes at the molecular level, so that they can be moledularly cloned from cellular clones of melanocytes that have lost their color. These genes are being cloned as markers for mapping and gene transfer that can be visualized in cell culture or newly hatched chicks. The Nebraska station will use ALV vectors developed by S.H. Hughes to attempt the transfer of pigment genes first somatically, but ultimately into the germ line.

29.026 CRIS0094649
GENETIC BASES FOR RESISTANCE AND IMMUNITY TO AVIAN DISEASES

TAYLOR R L JR; Animal & Nutrititional Science; University of New Hampshire, Durham, **NEW HAMPSHIRE** 03824.
Proj. No.: NH00303 Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 88 to 30 SEP 93

Objectives: Identify and characterize genes and their relationships to disease resistance in poultry with emphasis on MHC and linked genes as well as other sequences encoding cellular antigens, host cell receptors and other candidate systems. Identify, characterize and modulate environmental and physiological factors that regulate or affect immune system development, optimal immune function and disease resistance in poultry genetic stocks.

Approach: Continue production of 6.b and 003.R congenic chicken lines containing different MHC genes and recombinants. Test these lines to define MHC chromosomal regions contributing to antibody and cellular immune responses to Rous sarcoma virus and Eimeria tenella. Produce and characterize avian lymphokines interleukin-1 (IL-1) and interleukin-2 (IL-2). Test lymphokine production in genetic stocks to define specific associations of MHC and/or background genes with lymphokine production. Assess the effect of IL-1 and IL-2 treatment on Rous sarcoma regression. Study the effect of exogenous serotonin, dopamine and norepinephrine on the avian immune response and immune development. Examine in selected lines the influence of bursa of Fabricius size on immunity to S. aureus and E. tenella.

Progress: 88/01 to 88/12. Among line UNH 105 chickens segregating for B complex haplotypes, B22, B26 and B30, B22/B26 chickens had the greatest anti-tumor response which was significantly different from B26/B26 and B30/B30 chickens, the lowest responders. Lower mean TPI of two heterozygotes, B22/B26 and B26/B30 compared to appropriate homozygotes suggested complementation between B22 and B26 as well as B26 and B30. Immunization with five consecutive daily exposures of 500 E. tenella oocysts followed 21 days later by challenge with 10,000 oocysts reduced caecal lesion scores in BR5 (BF21-G19) and BR6 (BF21-G23) homozygous chicks compared to BR3 (BF2-G23) and BR8 (BF2-G2,23) homozygotes. These results suggest that the B-F chromosomal subregion imparts some control over the mechanism of acquired immunity to E. tenella. In three immunization protocols, recombinant coccidial antigen 3264 elicited a protective response in B5/B5 6 subline 1.B congenic chickens but not in B2/B2 chickens of the same line. Significant differences existed in both cecal lesion scores and weight gain. Fourteen days after immunization, B5/B5 chickens had significantly higher lymphocyte proliferation compared to B2/B2 chickens. In vitro migration of line UNH 105 peripheral blood leukocytes was augmented by 100 ng serotonin but suppressed by 1 ug serotonin. Dopamine suppressed PBL migration and norepinephrine enhanced migration.

Publications: 88/01 to 88/12

LUKACS, N., MCCORKLE, F.M. and TAYLOR, R.L., Jr. 1987. Suppression of the phytohemagglutinin wattle response by biogenic amines. Dev. Comp. Immunol. 11:759-768.

TAYLOR, R.L., JR., COTTER, P.F., WING, T.L. and BRILES, W.E. 1987. Major histo-compatibility (B) complex and sex effects on the phytohaemagglutinin wattle response. Anim. Genet. 18:343-350.

LUKACS, N. 1988. Response of major histocompatibility (B) complex haplotypes B22, B26 and B30 to Rous sarcoma and the role of endogenous lectins in avian Eimeria parasites. M.S. Thesis, University of New Hampshire, Durham. 68 p.

TAYLOR, R.L., JR. 1988. Regulation of immune responses to pathogens: T, B and MHC. In: Proc. National Breeder's Roundtable, I.Y. Pevzner, (ed.). Poultry Breeders of America, pp. 157-175.

TAYLOR, R.L., JR., CLARE, R.A., WARD, P.H., BRILES, R.W. and BRILES, W.E. 1988. Anti-Rous sarcoma response of major histocompatibility (B) complex haplotypes B23, B24 and B30. Anim. Genet. 19:277-284.

29.027 CRIS0034355

DEVELOPMENT OF A DNA PROBE FOR THE RAPID DETECTION OF MYCOPLASMA GALLISEPTICUM

GEARY S J; Bionique Laboratories Inc; Bloomingdale Road, Saranac Lake, **NEW YORK** 12983.

Proj. No.: NYK-8602401 Project Type: SMALL BUSINESS GRANT

Agency ID: SBIR Period: 15 SEP 86 to 30 SEP 89

Objectives: The objective of this project is to construct a specific Mycoplasma gallisepticum biotinylated DNA probe. Economic losses from M. gallisepticum infection are estimated to be $118 million annually in the United States. Maximum efficiency of M. gallisepticum disease control requires a rapid and sensitive identification system such as a probe. Total M. gallisepticum genome DNA was labeled with biotin-11-dUTP.

Approach: It is capable of detecting 75 ng of DNA or 7.5 x 10 broth-grown organisms by hybridization reaction in 19 hours. This probe also detects many other mycoplasmas. Mycoplasma gallisepticum species-specificity will be accomplished by cloning an M. gallisepticum specific DNA sequence into 2 Charon 4A and using this as the DNA source for the biotinylated probe.

Progress: 86/09 to 88/09. The cloning of EcoR1 fragments of M. gallisepticum DNA into bacteriophage 2 gt11 resulted in an efficiency of 3.4 X 10 recombinants per ug of M. gallisepticum DNA. Nitrocellulose (NTC) filter lift of clone plaques when hybridized with the biotinylated M. gallisepticum total genome DNA probe reacted with all of the clear plaques as expected. This verifies that plaques did contain M. gallisepticum DNA fragments. An identical NTC filter lift as hybridized with a biotinylated A. laidlawii total genome DNA probe. This indicated those plaques which contained DNA fragments that are at least in part identical to both M. gallisepticum and A. laidlawii. The plaques that hybridized only to the M. gallisepticum probe were propogated and their insert size determined. Lambda gt11-Mg6 was found to contain 5.5 Kb EcoR1 fragment. Southern blot hybridization using biotinylated lambda gt11-Mg6 5.5 Kb EcoR1 fragment as the probe shows the only species of mycoplasma recognized is M. gallisepticum. The quantity of DNA obtained by the extraction of the fragment from the low melting point agarose was insufficient to generate a strong hybridization signal so the 5.5 Kb fragment was subcloned into the plasmid pGEM-3Z and then designated pMg6. The total pMg6 vector was then biotinylated and utilized as a probe. 750 ng each of various mycoplasma EcoR1-digested DNAs were loaded per well in an agarose gel. After Southern blot transfer, the NTC was hybridized with 1 ug of pMg6 probe.

Publications: 86/09 to 88/09

GEARY, S.J. 1987. Development of a biotinylated probe for the rapid detection of Mycoplasma gallisepticum. Israel J. Med. Sci. 23:747-751.

GEARY, S.J., INTRES, R. and GABRIDGE, M.G. 1988. Species-specific biotinylated DNA probe for the detection of Mycoplasma gallisepticum. Mol. Cell. Probes 2:237-244.

GEARY, S.J., GLADD, M.F. and GABRIDGE, M.G. 1988. Species-specific biotinylated DNA probe for Mycoplasma gallisepticum. Zentralblatt for Bacteriologie, Microbiologie und Hygiene, "In Press.".

29.028 CRIS0134047

MOLECULAR EPIDEMIOLOGY OF AVIAN INFECTIOUS BRONCHITIS VIRUS AND APPLICATION OF OLIGONUCLEOTIDE

NAQI S A; PALUKAITIS P; Avian & Aquatic Animal Medicin; Cornell University, Ithaca, **NEW YORK** 14853.

Proj. No.: NYCV-426-537

Project Type: ANIMAL HEALTH

Agency ID: CSRS Period: 01 JAN 88 to 30 SEP 90

Objectives: Obtain oligonucleotide fingerprints of 2 serotypes of IBV, namely M 41 & ARK 99; sequence oligonucleotides that appear unique to each of the two serotypes; synthesize labeled oligonucleotides complimentary to the unique sequences of M 41 and ARK 99 genomes; evaluate the application of labeled oligonucleotides as diagnostic and epidemiological tools.

Approach: Two serotypes of avian infectious bronchitis virus will be analysed by comparing their RNA fingerprint profiles. Oligonucleotides unique to each of the two serotypes will be sequenced. Synthetic oligonucleotides complimentary to the unique sequences of the two IBV serotypes will be made & used as diagnostic and epidemiological tools. Since the complete nucleotide sequence of one strain of IBV is now known, sequence information generated in this study will allow precise understanding of both the nature and loci of sequence variation among these

serotypes.

Progress: 88/01 to 88/12. Oligonucleotide fingerprints of the two serotypes of IBV (M 41 and ARK 99) were obtained. These patterns were distinctly different. In addition, fingerprints of a third distinctive IBV serotype (Conn 46) was obtained. Fingerprints of the above IBV isolates were compared with three isolated from enteric cornaviruses (DCV) isolated in New York State. From the fingerprint patterns, the three ECV isolates appear to be more related to each other than to the three IBV isolates. That these three ECV isolates do in fact contain extensive nucleotide sequence homology with the IBV isolates was shown by nucleic acid hybridization. Complementary DNA (cDNA) prepared to the viral RNA of the M 41 isolate of IBV, hybridized to nucleic acids from the other two IBV isolates, as well as to the three ECV isolates, but not to RNA of tobacco mosaic virus. These results and the serological data (virus neutralization) obtained in our laboratory show that the ECV isolates are probably strains of IBV exhibiting different tissue tropism. Nucleotide sequence analysis of oligonucleotides of the three ECVs should provide some indication of the degree of similarity of these strains to the published IBV nucleotide sequence.

Publications: 88/01 to 88/12

NAQI, S.A., LUCIO, B., KARACA, K., and PALUKAITIS, P. 1988. Characterization of three intestinal isolates of chicken coronavirus. (Abstract). 60th Northeastern Conference on Avian Diseases, June 13-14, Mystic, CT.

NAQI, S.A., LUCIO, B., KARACA, K. and PALUKAITIS, P. 1988. Characterization and pathogenicity of intestinal isolates of coronavirus in normal and IgA deficient chickens. (Abstract). 125th Annual AVMA Meeting, July 17-21, p. 136.

29.029 CRIS0137234

GENETIC VARIATION AMONG DUCK HEPATITIS VIRUS TYPE I STRAINS EXAMINED BY O

WOOLCOCK P R; Avian & Aquatic Animal Medicin; Cornell University, Ithaca, **NEW YORK** 14853.

Proj. No.: NYCV-426-312
Project Type: ANIMAL HEALTH
Agency ID: CSRS Period: 13 JAN 89 to 30 SEP 91

Objectives: Detection of molecular variation among wild, attenuated and variant duck hepatitis virus type I isolates.

Approach: Molecular variation will be detected at the genome level by oligonucleotide mapping of viral RNA and the gene product level by SDS-PAGE of viral proteins.

29.030 0083564

GENETIC BASES FOR RESISTANCE AND IMMUNITY TO AVIAN DISEASES

BLOOM S E; DIETERT R R; MARSH J A; Poultry & Avian Sciences; Cornell University, Ithaca, **NEW YORK** 14853.

Proj. No.: NYC-157433 Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 88 to 30 SEP 91

Objectives: Identify and characterize genes and their relationship to disease resistance in poultry with emphasis on the major histocompatibility complex (MHC) and linked genes such as the ribosomal RNA cluster. Identify and characterize genetic and physiologic factors that modulate optimal immune development, immune function, and disease resistance using poultry strains. Develop methodologies and reagents to assess immune function on disease resistance for the purpose of enhancing production efficiency through genetic selection in poultry.

Approach: Chickens differing in the number of MHC copies will be bred and tested for levels of resistance to several pathogenic viruses and parasites in special isolation units. MHC copy number variants will also be used to study the influence of this region on performance traits. The expression of surface antigens on lymphocytes (Ia) and erythrocytes (B-G) will be studied in chickens having altered MHC copies including segregants of the trisomic strain and R recombinants (B-G region variants). Immune development and functions will be evaluated in chickens having altered Ia expression to test for the effects of varying amounts of this product. The use of trisomic chickens for enhancing recombination within the MHC and neighboring ribosomal RNA genes will be explored.

Progress: 88/01 to 88/12. Studies aimed at manipulating the major histocompatibility complex (MHC) and the tightly linked ribosomal genes (rDNA) are being performed with the aim of enhancing performance including disease resistance in genetic strains and crosses. Our previous work with trisomic matings indicates that unequal recombination in rDNA may be enhanced in meiosis involving trivalents. We, therefore, tested for enhanced unequal recombination within the MHC in trisomic matings. The R8 chromosome was used as the marker. The uncoupling of the G2-G23 duplicated region would result in G2G15 and G23G15 genotypes (instead of G2G23G15). No such recombinants were detected among 178 progeny subjected to intensive typing studies. In the same experiment, 5% of progeny showed evidence of unequal recombination for rDNA. In crosses of rDNA variants, we found one family transmitting an extreme alteration in nucleolar phenotype in offspring. The B6 chromosome may possess an altered rDNA genotype that enhances unequal recombination or expression of rRNA gene clusters. Despite the presence of a minute nucleolus, two haplotypes were expressed (i.e., B6B15). Thus, severe changes at rDNA cluster did not apparently alter MHC expression. Thus, the G2-G23 duplicated segment (R8) of the MHC was inherited as a stable gene block in crosses designed to enhance unequal recombination. In

contrast, variation in rDNA content was induced in one generation. Such variants will be useful in selecting for enhanced growth and diseases resistance.

Publications: 88/01 to 88/12

BLOOM, S.E. 1988. Gene expression chickens aneuploid for the MHC-bearing chromosome. pp 1-20. In: The molecular biology of the major histocompatibility complex. Eds. C.M. Warner, et al., Iowa State University Press.

DELANY, M.E., DIETERT, R.R. and BLOOM, S.E. 1988. MHC-chromosome dosage effects: Evidence for increased expression of Ia glycoprotein and alteration of B cell subpopulations in neonatal aneuploid chickens. Immunogenetics 27:24-30.

GOTO, R., MIYADA, C.G., YOUNG, S., WALLACE, R.B., ABPLANALP, H., BLOOM, S.E., BRILES, W.E. and MILLER, M.M. 1988. Isolation of a cDNA clone from from the B-G subre gion of the chicken B-complex. Immunogenetics 27:102-109.

GREENFIELD, C.L., SANDERS, F.S. and DIETERT, R.R. 1988. Detection of avian macrophages with Concanavalin A. In press. Avian Pathol.

QURESHI, M. A., DIETERT, R.R. and BACON, L.D. 1988. Chemotactic activity of chicken blood mononuclear leukocytes from 15I5-B-congenic lines to bacterially- derived chemoattractants. In press. Vet. Immunol. Immunopathol.

29.031 CRIS0092637

INTRODUCTION OF NEW GENETIC MATERIAL INTO AVIAN EMBRYOS

BLOOM S E; VOGT V M; MARSH J A; Poultry & Avian Sciences; Cornell University, Ithaca, **NEW YORK** 14853.

Proj. No.: NYC-157306 Project Type: STATE
Agency ID: SAES Period: 01 JAN 84 to 30 JUN 87

Objectives: To explore methods of introducing DNA into the germ line of chickens. To utilize the techniques developed in objective one to bring about transformation with genes that may affect the economic potential and disease resistance. To study the organization and expression of midosomal RNA sequences in chickens having varying chromosome complements. To study the affects of growth hormone on growth potential and immune system development.

Approach: DNA representing defined sequences will be injected into chicken cells in vivo and in vitro. Chickens will be assayed at selected stages for integration into the host chromosome and the expression of such sequences. The gene copy number will be determined for diploid chickens and in those birds having additional ribosomal DNA-containing chromosomes. Chickens containing extra genetic material will be studied for possible increased gene expression and products. The effects of such additional gene product on growth, development, and functions of the immune system will be studied. The biological effects of various forms of growth hormone will be studied in genetically defined chickens to better understand the role of this hormone in growth and development. These systems will contribute to the understanding of the role of specific genes and gene products in various aspects of development and immune function.

Progress: 87/01 to 87/12. The genes encoding 18S, 5.8S, and 28S ribosomal RNA (rRNA) are tandemly repeated at the nucleolus organizer region (NOR). A line of chickens that contains individuals that are either disomic, trisomic, or tetrasomic for this chromosome has been developed. Aneuploid animals display a proportional increase in the rRNA gene copy number per cell, but the amounts of mature rRNA are regulated to normal levels. This study addressed the question of how regulation of mature rRNA synthesis occurs in aneuploid cells. An analysis of rRNA transcription in chicken embryo fibroblasts (CEFs) revealed that the relative rates of rRNA synthesis and processing and the amounts of precursor rRNA per cell were similar for all three genotypes. A comparison of chromatin structure, as determined by sensitivity of rDNA in nuclei from CEFs to digestion by DNase I, revealed that some of the rRNA genes from aneuploid cells were more resistant to digestion than corresponding sequences in the disomic cells. A determination of the distribution of topoisomerase I on rDNA was performed using the compound camptothecin, which introduces breaks in topoisomerase-DNA complexes. Quantitation of camptothecin-induced cleavages revealed that a larger proportion of the rRNA genes in aneuploid cells were resistant to cleavage than in disomic cells, and therefore have no detectable amounts of topoisomerase I.

Publications: 87/01 to 87/12

MUSCARELLA, D.E., VOGT, V.M., and BLOOM, S.E. 1987. Characterization of ribosomal RNA synthesis in a gene dosage mutant: The relationship of topoisomerase I and chromatin structure to transcription. J. Cell Biol. 105:1501-1513.

ERF, G.F. and MARSH, J.A. 1987. Cell-mediated immunity in relation to thyroid hormone status. Poult. Sci., In press.

ERF, G.F. and MARSH, J.A. 1987. Triiodothyronine affects on mitogen responsiveness in sex-linked dwarf and Cornell K strain chickens. Devel. Comp. Immunol., In press.

LEUNG, F.C., STYLES, W.J., ROSENBLUM, C.I., LILBURN, M.S. and MARSH, J.A. 1987. Diminished hepatic growth hormone receptor binding in sex-linked dwarf broiler and leghorn chickens. Proc. Soc. Exp. Biol. Med. 184:234-238.

29.032 CRIS0096209

CLONING AND EXPRESSION OF NEWCASTLE DISEASE VIRUS SURFACE PROTEINS

DEBUYSSCHER E V; FULLER F; Microbiology Path & Parasito; North Carolina State University, Raleigh, **NORTH CAROLINA** 27695.

Proj. No.: NC09054 Project Type: SPECIAL GRANT
Agency ID: CSRS Period: 01 AUG 85 to 31 JUL 89

Objectives: Identification of the cloned NDV cDNA's that contain homologous sequences for the HN and F mRNA's. Synthesis of ds cDNA of the complete mRNA's for coding for the HN and F. proteins.

Approach: The goal is to insert the genes coding for the HN and F proteins of NDV in a non-pathogenic poultry virus to induce protective immunity. First, an NDV-genomic library will be constructed. Second, the cloned cDNA's coding for F and HN proteins will be identified by colony hybridization. Complete mRNA's for HN and F proteins will be isolated by hybridization selection. Third, cDNA copies of the complete HN and F mRNA's will be linked to a promotor sequence and inserted in the genome of a non-pathogenic avian ds DNA virus.

Progress: 88/01 to 88/12. Recently we determined the sequence of the F-gene of strain Texas G.B. The sequence data were obtained for the two full or near full length cDNA clones (pF135 and pF139) of the F. mRHA. Fig. 3 shows the nucleotide and the deduced AA sequence for the F-gene. The sequence at the cleavage site RRQKR F (amino acids 112-117) matches the consensus sequence at this site to all virulent NDV strains (RRQK/RR F) examined today. When a comparison is made between the AA sequence of Texas G.B. and the closely related Beaudette C fusion gene, there are only two differences found: the first at position 11 (valine vs. threonine) and the 2d at position 197 (leucine vs. serine). We are uncertain at this point what role, if any, the HN and F of the Texas G.B. strain play in the virulent and neutrotopic properties of this virus. A comparison of the envelope proteins of the closely related Beaudette C. reveals 11 AA differences between them in NH and two in F. Since both strains contain the same F. cleavage site, it is hard to relate these small differences in F to the observed differences in pathogenicity. At this point we suggest that these differences in pathogenicity are more likely due to the more numerous coding differences for the NH-protein. Using the Chou-Fasman prediction model, we developed tentative maps of the secondary structures for the NH-and F-proteins in order to determine important antigenic sites on these proteins.

Publications: 88/01 to 88/12

WARD, M.D.W., 1988. The characterization of Newcastle Disease Virus Gene Clones. M.S. Thesis, North Carolina State University.

SCHAPER, V. et al: 1988. Nucleotide sequences of the envelope protein genes of a highly virulent, neurotypic strain of Newcastle Disease Virus. Virology 165:291-296.

29.033 CRIS0133846

MOLECULAR CLONING OF GENES ESSENTIAL FOR ANTIGENICITY OF INFECTIOUS BURSAL DISEASE VIRUSES

JACKWOOD D J; SAIF Y M; Food Animal Health Research; Ohio State University, Wooster, **OHIO** 44691.

Proj. No.: OH000895 Project Type: HATCH
Agency ID: CSRS Period: 01 DEC 87 to 31 OCT 91

Objectives: Prepare molecular clones of genes from a serotype I and serotype II IBDV. Determine the nucleotide sequences of cDNA clones from the large RNA genome segment which codes for structural viral proteins. Express the major structural viral proteins using recombinant DNA techniques and examine their immunogenicity and potential as diagnostic reagents.

Approach: The genome of IBDV will be extracted from viral particles and used as a template for the synthesis of cDNA. The cDNA molecules will be cloned into the plasmid pUC-9. The molecular sequence of clones containing structural protein genes will be determined using the dideoxy chain termination technique. Selected open-reading-frames will be subcloned into various expression vectors containing eukaryotic promoters. The immunogenicity and diagnostic potential of the expressed proteins will be studied in SPF chicks.

Progress: 88/01 to 88/12. Molecular clones were prepared from the structural protein genes (genome segment A) of three Infectious Bursal Disease Viruses; a virulent serotype 1 virus (ST-C), an attenuated serotype 1 virus (BB), and a serotype 2 virus (OH). Selected cDNA clones were determined to contain viral specific sequences using the northern blot hybridization assay. Colony blot hybridization were used to identify related clones among the three viruses. The nucleotide sequences of selected clones were determined from cDNA in the original cloning vector, pUC9, using a modification of the dideoxy-chain termination procedure. Sequence homology among the viruses was determined for portions of the VP-2, VP-3, and VP-4 genes. A high degree of nucleotide sequence homology (>90%) was observed over most of the regions sequenced. However, some regions exhibited more variability than others.

Publications: 88/01 to 88/12

JACKWOOD, D.J. 1988. Detection of infectious bursal disease virus using nucleic acid probes. Abstr. 125th AVMA Meet. 195.

JACKWOOD, D.J. 1988. Development and characterization of nucleic acid probes to infectious bursal disease viruses. Abst. from Nucleic Acid Probes in Vet. Infectious Diseases, Proc. Third Workshop, American College of Vet. Microbiologists.

JACKWOOD, D.J., KIBENGE, F.S.B. and MERCADO, C.C. 1988. Nucleotide sequence comparisons of the structural protein genes among infectious bursal disease viruses. 69th Conference of Research Workers in Animal Diseases, Chicago, Ill., KIBENGE, F.S.B., MERCADO, C.C. and JACKWOOD, D.J. 1988. Comparison of nucleotide sequences of the polymerase gene of four infectious bursal disease viruses. 69th Conference of Research Workers in Animal Diseases, Chicago, Ill.

JACKWOOD, D.J. 1988. The use of nucleic acid probes to detect viral infections in poultry. Ohio Turkey Days, Proceedings.

29.034 CRIS0095620
REGULATION, EXPRESSION AND STRUCTURAL ORGANIZATION OF THE CHICKEN GROWTH HORMONE GENE

FOSTER D N; Poultry Science; Ohio State University, Wooster, **OHIO** 44691.
Proj. No.: OH000800 Project Type: HATCH
Agency ID: CSRS Period: 01 JUN 85 to 31 MAY 89

Objectives: To clone the chicken growth hormone (cGH) gene from a genomic library. To partially characterize the structure of cGH gene. To determine what regulates cGH gene expression at the molecular level. To see how various physiological parameters affect cGH transcription in vivo. To determine the levels of cGH induction and accumulation. To find out if increased levels of cGH protein are the result of increased transcription or enhanced mRNA stability.

Approach: A rat growth hormone cDNA clone (Seeburg et al., Nature 270, 486-494) will be used as a heterologous probe to isolate the chicken growth hormone gene from a chicken oviduct genomic library. The structural organization of the cloned gene will be accomplished by restriction endonuclease mapping. Various environmental and physiological parameters will be tested to determine the extent of inducibility and expression of cGH specific transcripts by northern blot analysis. Increased levels of cGH protein may depend on increased transcription or increased message stability. The levels of accumulation of cGH mRNA transcripts will be measured by northern blot analysis and the t1/2 of the cGH mRNA will be measured by the methods of Yaffe and Samuels (J. Biol. Chem. 259, 6284-6291).

Progress: 88/01 to 88/12. Several positive thyroid stimulating hormone (TSH) beta cDNa clones have been isolated from a chicken pituitary hormone cDNA library using a heterologous bovine TSH beta cDNA probe. The purified cloned cDNAs were digested with EcoR1, subjected to agarose gel electrophoresis, Southern blotted and probed with the bovine TSH beta probe. Four of the cDNAs hybridized to the probe suggesting the chicken clones were TSH beta specific. The TSH beta clones contained approximately 500 bp inserts and were subcloned into the EcoR1 site of the sequencing vector M13 mp18 for subsequent nucleotide sequence analysis. One of these subcloned inserts (TSH beta-4) was sequenced from the 3' end of the clone. Of the 320 bp sequenced, no apparent homologous match was noted. It is very possible that the 320 bp sequenced were all in the 3' untranslated region where a high degree of evolutionary divergence would occur and therefore yield a low degree of cross-species homology. Sequence analysis of the remainder of TSH beta-4 is ongoing.

Publications: 88/01 to 88/12
LAMB, I.C., GALEHOUSE, D.C. and FOSTER, D.F. 1988. Chicken growth hormone cDNA sequence. Nucleic Acids Res. 16:9339.
LAMB, I.C., GALEHOUSE, D.C., BACON, W.L. and FOSTER, D.N. 1988. Chicken growth hormone cDNA: isolation, cloning and sequence analysis. Poultry Sci. 67:107.

29.035 CRIS0138462
CHICKEN GROWTH HORMONE GENE REGULATION

FOSTER D N; Poultry Science; Ohio State University, Wooster, **OHIO** 44691.
Proj. No.: OH000966 Project Type: HATCH - PENDING
Agency ID: CSRS Period: 01 JUL 89 to 30 JUN 94

Objectives: To clone and determine the nucleotide sequence of the cGH gene. To quantify cGH transcription levels and rates from nuclei of cultured pituitary cells. To fine map the 5'-portion of the cGH gene by deletion mutation analysis.

Approach: A cGH full length cDNA will be used to isolate the cGH sequence from a genomic library. Nucleotide sequence analysis will allow the structural organization to be elucidated. Fine map deletion mutation analysis will be used to determine the cis-acting sequences responsible for regulating the gene. The accumulation of cGH mRNA and the specific rates of cGH transcription will be measured by northern blot analysis and nuclear run-off assays, respectively, after primary pituitary cultures have been treated with effectors of cGH transcription.

29.036 CRIS0097073
ISOLATION AND STRUCTURAL ORGANIZATION OF THE CHICKEN GONADOTROPIC GENES

FOSTER D N; BACON W L; Poultry Science; Ohio State University, Columbus, **OHIO** 43210.
Proj. No.: OH000391-SS Project Type: CRGO
Agency ID: CRGO Period: 01 SEP 85 to 31 DEC 88

Objectives: Proj 8502858. To better understand the molecular mechanisms of how the chicken pituitary gonadotrophic luteinizing hormone (LH) affects growth, reproduction and metabolism. To clone and characterize the chicken LH alpha and beta genes from genomic and pituitary cDNA/expression libraries. To characterize the size of the transcribed mRNA's and to analyze their expression.

Approach: The genes from the alpha and beta subunits of chicken pituitary LH will be isolated from a chicken pituitary cDNA/expression library. The constructed library will be screened using LH specific antisera. Another screening approach will utilize heterologous mammalian LH cDNA probes. After the isolation and cloning of the putative LH genes, their identity will be verified by hybrid selection techniques followed by in vitro translation and gel electrophoresis of the gene product. The structural organization of the LH genes will be analyzed by restriction mapping, nucleotide sequencing and exon/intron characterization. The number of genomic alpha and beta LH sequences will be determined by Southern blot analysis and the size of the mRNA transcripts will be determined by Northern blot analysis.

Progress: 85/09 to 88/12. This project sought to isolate and clone the genes that encode the chicken pituitary alpha and luteinizing hormone (LH) beta proteins. We prepared a cDNA expression library utilizing poly A+ mRNA from adult layers and screened this library with a heterologous bovine alpha cDNA probe (R. Maurer, University of Iowa). We isolated a 320 bp cDNA clone that encoded the entire 96 amino acid protein of alpha but lacked both 5' and 3' regions of the full length cDNA. We prepared a second cDNA library using mRNA from the pituitaries of 7 week old broilers. The pituitaries were superfused and given chicken GnRH prior to processing the organs for RNA isolation. We used our homologous 320 bp chicken alpha cDNA to screen this library and generated two alpha specific probes. Nucleotide sequence analysis revealed a 709 bp cDNA that contained all of the alpha coding region (including the 24 amino acid leader polypeptide) plus 5' and 3' untranslated regions plus a 45 bp poly A tract. The chicken alpha mRNA transcript appears to be about 800 bp as determined by northern blot analysis, is specifically upregulated after stimulation with shicken GnRH, and is tissue specific in nature (the alpha cDNA hybridizes to chicken pituitary RNA but not to chicken liver RNA). Several putative chicken LH beta cDNA clones are currently being characterized by nucleotide sequence analysis. We have used chicken specific LH RIA to measure the amounts of secreted LH upon GnRH treatment in a superfusion system.

Publications: 85/09 to 88/12
NO PUBLICATIONS REPORTED THIS PERIOD.

29.037 CRIS0088380
ADVANCED TECHNOLOGIES FOR THE GENETIC IMPROVEMENT OF POULTRY

NESTOR K E; FECHHEIMER N S; FOSTER D N;
Poultry Science; Ohio State University,
Columbus, **OHIO** 43210.
Proj. No.: OH000740 Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 87 to 30 SEP 92

Objectives: Develop methods for creating new genetic variation in poultry by gene transfer and chromosome alteration. Develop more efficient selection methods, including the utilization of genetic variation introduced by new technologies, for improving the economic traits of poultry.

Approach: Procedures aimed at producing triploidy by suppression of the second polar body division will be tested in both males and females. When a successful treatment is found, eggs will be incubated to determine the viability of triploids. Survival of progeny bearing chromosomal abberations will be assessed. Current long term selected turkey lines (E and F) along with their randombred controls will be maintained. A new randombred control population of turkeys will be developed by crossing the F line with a commercial growth line. Eight Japanese quail lines, a randombred control, lines divergently selected for body weight (HW, LW), plasma yolk precursor (HP, LP), and a combination of these traits (HW-HP, HW-LP, LP-HW) will be continued. Growth, reproduction, and various physiological parameters will be measured in the above lines.

Progress: 88/01 to 88/12. A chicken pituitary cDNA library (constructed from poly A+ mRNA isolated from chicken pituitaries that had been placed into a superfusion chamber and given 10 nM chicken GnRH 1 for 30 minutes) was screened to isolate a full length chicken growth hormone (cGH) sequence. A heterologous bovine growth cDNA probe was used to screen the cDNA library. A number of putative cGH cDNA clones were selected and subjected to subsequent rounds of purification. The insert from several plaque purified clones were subcloned into the plasmid vector pUC118 and grown in mass. Two of the putative cGH clones were nucleotide sequenced. The primary sequence for the cloned cGH was 774 nucleotides long. The cloned cDNA nucleotide sequence contained 42 base pairs (bp) of untranslated region (UT) at the 5' end of the molecule, and 81 bp of UT at the 3' end plus a poly A tail. The cDNA encodes a leader poly peptide for 25 amino acids plus a 191 amino acid mature cGH protein. The cGH shares 76% homology to a recently cloned duck GH. There is a putative glycosylation site near the carboxy terminus of the protein which could be the most important in conferring biological activity. The cGH cDNA that has been isolated shows tissue specificity. Slot blot analysis of total cellular RNA isolated from chicken pituitary and liver tissues probed with radioactivity labeled cGH showed that the probe specifically hybridized to the pituitary RNA.

Publications: 88/01 to 88/12
HAVENSTEIN, G.B., NESTOR, K.E. and BACON, W.L. 1988. Comparison of pedigreed and nonpedigreed randombred control systems for use with artificial selection in the Japanese quail. Poultry Sci. 67:357-366.
BLATTI, S.P., FOSTER, D.N., RANGANATHAN, G., MOSES, H.L. and GETZ, M.J. 1988. Induction of fibronectin gene transcription and mRNA is a primary response to growth-factor stimulation of AKR-2B cells. Proc. Natl. Acad. Sci. USA 85:1119-1123
MIN, G.-H., GIORDANO, T., STRAUCH, A.R. and FOSTER, D.N. 1987. Isolation and partial characterization of a murine vascular smooth muscle alpha-actin cDNA. J. Cell Biol. 105:26a.
GIORDANO, T. and FOSTER, D.N. 1988. Isolation and identification of a senescent-specific cDNA from WI-38 cells. In vitro 24:62A.
LAMB, I.C., GALEHOUSE, D.C., BACON, W.L. and FOSTER, D.N. 1988. Chicken growth hormone cDNA: Isolation, cloning and sequence analysis. Poultry Sci. 67:107.
LILBURN, M.S., NESTOR, K.E. and STAMP, L.M. 1988. Developmental characteristics of turkey genotypes selected for body weight gain and muscle development. Poultry Sci. 67:22.
LILBURN, M.S., NESTOR, K.E. and STAMP, L.M. 1988. Selection for body weight at 4 weeks and its relationship with the protein requirement of Japanese quail from 0-4 weeks. Poultry Sci. 67:22.

29.038 CRIS0130042
REGULATION OF THE AMOUNT OF BODY FAT IN POULTRY

GOODRIDGE A; Western Reserve University, Cleveland, **OHIO** 44106.
Proj. No.: OHOR-8601244 Project Type: CRGO
Agency ID: CRGO Period: 15 AUG 86 to 31 AUG 90

Objectives: Project 8601244. The objective of this proposal is to "construct", using recombinant DNA techniques, a strain of poultry which contains less body fat.

Approach: We plan to insert an anti-gene for acetyl-CoA carboxylase into the germ line. Expression of this anti-gene should inhibit expression of the natural gene which, in turn, should block conversion of carbohydrate to fat.

Progress: 86/01 to 87/08. A Japanese group reported the isolation of cloned DNA for chicken acetyl-CoA carboxylase. We have prepared a synthetic oligomer of 40 nucleotides based on the published sequence. We are in the process of screening a chicken liver cDNA library (lambda gt 11). If this screening is negative, we will prepare our own cDNA library in the lambda gt 10 vector, using RNA which we know to have induced levels of acetyl-CoA carboxylase mRNA. A 5′ restriction fragment of the malic enzyme cDNA (PstI to HindIII, 440 bases) has been cloned into an expression vector. We substituted the malic enzyme sequences for the chloramphenicol acetyl transferase (CAT) gene in pRSVCAT. The powerful promoter/enhancer of the Rous Sarcoma Virus (RSV) LTR drives transcription in these vectors. Clones containing the malic enzyme cDNA in each orientation were isolated. pRSVME440+ and pRSVME 440- were co-transfected into quail QT cells wit pRSVNeo, a construction which expresses the bacterial gene for resistance to the antibiotic G418. Resistant colonies of cells have been selected and are being expanded. Chicken embryo fibroblasts and quail QT6 cells have low levels of malic enzyme activity which are not affected by hormones and should be appropriate for testing the effect of our anti-sense genes. We are also characterizing some intron-specific clones isolated from the cloned gene for chicken malic enzyme.

Publications: 86/01 to 87/08
NO PUBLICATIONS REPORTED THIS PERIOD.

29.039 CRIS0130713
STRUCTURAL ORGANIZATION AND REGULATION OF THE CHICKEN THYROID STIMULATING HORMONE GENES

FOSTER D N; BACON W L; Poultry Science; 1314 Kinnear Rd, Columbus, **OHIO** 43212.
Proj. No.: OHO00407-SS Project Type: CRGO
Agency ID: CRGO Period: 01 SEP 86 to 31 AUG 89

Objectives: Proj. 8601351. To eluoidace the molecular mechanisms of how the chicken pituitary thyroid stimulating hormone (TSH) affects growth, reproduction and metabolism. To clone, identify, and determine the structural organization of the chicken alpha and TSH-Beta genes from both chicken genomic and pituitary cDNA/expression libraries. To determine intron/exon position and number, the start site of transcription, and the copy number of the alpha and TSH-Beta sequences.

Approach: Chicken alpha and TSH-Beta sequences are currently being isolated from chicken genomic and pitutary cDNA/expression libraries using heterologous mammalian cDNA probes. A putative genomic clone has been isolated and subcloned into pUC12 and will be restriction mapped before being sub-cloned and sequenced in the vector M13. A putative cDNA clone has been isolated, and cloned into M13 and is currently being sequenced. The gene products of these isolated clones will be analyzed by Western blots. The structural organization of the alpha and TSH-Beta genes will be characterized by further restriction mapping, confirmatory nucleotide sequencing (which will also determine the start site of transcription) and exon/intron number. The copy number of the alpha and genes will be determined by Southern blot analysis.

Progress: 88/01 to 88/12. Several positive thyroid stimulating hormone (TSH) beta cDNA clones have been isolated from a chicken pituitary hormone cDNA library using a heterologous bovine TSH beta cDNA probe. The purified cloned cDNAs were digested withEcoR1, subjected to agarose gel electrophoresis, Southern blotted and probed with the bovine TSH beta probe. Four of the cDNAs hybridized to the probe suggesting the chicken clones were TSH beta specific. The TSH beta clones contained approximately 500 bp inserts and were subcloned into the EcoR1 site of the sequencing vector M13 mp18 for subsequent nucleotide sequence analysis. One of these subcloned inserts (TSH beta-4) was sequenced from the 3′ end of the clone. Of the 320 bp sequenced, no apparent homologous match was noted. It is very possible that the 320 bp sequenced were all in the 3′ untranslated region where a high degree of evolutionary divergence would occur and therefore yield a low degree of cross-species homology. Sequence analysis of the remainder of TSH beta-4 is ongoing.

Publications: 88/01 to 88/12
NO PUBLICATIONS REPORTED THIS PERIOD.

29.040 CRIS0137536
FUNCTIONAL STUDIES OF RNA VIRAL POLYMERASES

COLLISSON E W; IRVIN R; Veterinary Microbiology & Parasitology; Texas A&M University, College Station, **TEXAS** 77843.
Proj. No.: TEX06978 Project Type: ANIMAL HEALTH
Agency ID: CSRS Period: 20 FEB 89 to 30 SEP 90

Objectives: Our long-range goal is to develop antiviral drugs which target the uniquely viral function of RNA-dependent RNA viral polymerases. The overall objective of this research project is to characterize the basic molecular biology of the RNA-dependent RNA polymerase of IBV, a single stranded RNA virus.

Approach: We will identify the viral-specific RNA binding polymerase of IBV in infected cells by assaying for decreases in the mobility of the 3' end of the IBV genome - 1. Fractions from infected cell lysates will be separated by charge or size using column chromatography and assayed for binding activity; 2. The polymerase proteins will be purified with specific antibodies made by immunizing chickens with synthetic oligopeptrides constructed from nucleotide sequencing data. Determine the nucleotide sequence requirements for polymerase binding. Binding sites will be localized by digesting the peptide nucleotide complex with nuclease and sequencing the resistent oligonucleotide. Develop an in vitro RNA transcription assay for the IBV polymerase to assay for enzyme function. Use the assay in vitro to examine anti-virql polymerase activity of various drugs.

29.041 CRIS0099060
MOLECULAR STUDIES OF VIRAL INDUCED AVIAN DISEASES

KEMP M; Veterinary Microbiology & Parasitology; Texas A&M University, College Station, **TEXAS** 77843.
Proj. No.: TEX06840 Project Type: HATCH
Agency ID: CSRS Period: 01 AUG 86 to 30 JUL 91

Objectives: Biochemical and genetic mapping to the genomic segments of avian reoviruses responsible for causing viral arthritis syndrome (VAS), malabsorption syndrome (MAS) and/or rapid death syndrome (RDS), Genetic reassortment studies to define the genomic segments and/or viral proteins involved in determining viral tropism and virulence, production of monoclonal antibodies (mABs) to be used in evaluating the immunological relationships of the avian reoviruses, determination of whether non-neutralizing epitopes are involved in defining the tropism of the virus and the resultant pathogenic properties.

Approach: Determine the proteins encoded by the avian reovirus genome segments by in vitro transcription of each segment followed by hybridization to total genomic RNA. Alternatively, mRNA transcripts will be correlated with genome segments by oligonucleotide fingerprinting studies. Having determined which segment codes for which mRNA, we will then determine which proteins are encoded by which mRNA by carrying out in vitro translation experiments. When these studies are complete we will use the mapping studies to define the factors involved in determining virulence and tropism. Genomic segments coding for certain proteins will be reassorted and the reassortant viruses will be tested by infecting broiler chicks.

Progress: 88/01 to 88/12. Avain reoviruses are the demonstrated causative agents of a variety of diseases in chickens. Comparison of the genomes of six selected avain reoviruses showed that the genomic segements of these viruses can be separated into three size classes. The electrophoretic mobilities of the genomic segment of each viral strain relative to those of the other isolates is highly variable, i.e. the genomes are highly polymorphic. The polymorphic nature of these viruses was highlighted by a pairwise comparison of the genomic segments and the encoded polypeptirdes, which showed that there are different degrees of genetic relatedness between the six selected isolates. While the viruses appear to be genetically dissimilar preliminary studies indicate that a number of the epitopes associated with the outer capsid proteins are conserved. In addition to the characterization of the genotypes and the proteins encoded by the six isolates we have determined the pathogen of each isolate. A direct correlation between a specific genotype and a particular pathotype has not been demonstrated but two distinct pathotypes have been demonstrated. All members of group I have been shown to cause tenosynovitis and to a lesser extent hepatomegaly, when administered via the footpad route. Members of group II exhibit a variable capacity to induce tenosynovitis when administered via the footpad route, but all members of this group induce a transient digestive system disorder when adminstered orally.

Publications: 88/01 to 88/12
CLARK, F.D., COLLISSON, E.W. and KEMP, M.C. Characterization of Avain Reovirus Strain-Specific Polymorphisms (Submitted to Journal of General Virology).

29.042 CRIS0001767
ADVANCED TECHNOLOGIES FOR THE GENETIC IMPROVEMENT OF POULTRY

BITGOOD J J; Poultry Science; University of Wisconsin, Madison, **WISCONSIN** 53706.
Proj. No.: WIS00726 Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 87 to 30 SEP 92

Objectives: Identify, characterize, and map poultry genes by molecular and classical methods. Develop and test procedures to apply emerging technologies to poultry breeding by theoretical modeling, simulation, and animal testing.

Approach: Objective A. Morphological mutations and cytologically marked chromosomes will be used in linkage studies to further develop the linkage map. Tissue samples for molecular studies will be provided to other stations. Comparisons between known carriers of selected mutations and normal individuals will be made in order to characterize the effects of the mutation. Objective B. As marked chromosomal regions are established, associations between these regions and selected economic traits will be evaluated. This will be done using classical backcross procedures.

Progress: 88/01 to 88/12. Gene mapping in the chicken continues. Studies of chromosome one are underway using pea comb, naked neck, tardy feathering, yellow skin, the NM 7092 translocation, and blood groups C, D, I and P

(in collaboration with Dr. Briles, Northern Illinois). Blood samples of the grandparents, parents and test cross progeny are being provided to Dr. Guise, MN, for DNA analysis. There still is no evidence to verify assignment of naked neck to chromosome 1 as shown on current maps. The linear order of 4 markers on the long arm of the Z chromosome was shown to be barring, dermal melanin inhibitor, the MN t(Z;1) break point, and recessive white skin, with the latter closest to the centromere. Pirouette was shown to segregate independently from pea comb, creeper, yellow skin and multiple spur. A new mutation was recovered that initially appears to be an allele at the recessive white feather color locus. Reciprocal crosses between Anconas and Leghorns to study shell color inheritance resulted in the female offspring producting tinted eggs. Back cross birds are being studied.

Publications: 88/01 to 88/12

ATTIE, A.D., POERNAMA, F., CHECOVICH, W.J., SCHREYER, S.A., BITGOOD, J.J. and COOK, M.E. 1988. Hypoalphalipoproteinemia associated with a Z-linked mutation in a strain of chicken. Eighth International Symposium on Atherosclerosis, Rome.

BITGOOD, J.J. 1988. Linear relationship of the loci for barring, dermal melanin inhibitor, and recessive white skin on the chicken Z chromosome. Poultry Sci. 67:530-533.

JOHNSON, T., BITGOOD, J.J. and MAURER, A.J. 1988. Effect of the am gene on percent abdominal fat pad and yield in a synthetic broiler line. Poultry Sci. 76(Suppl. 1):102.

30.001 CRIS0097564
MOLECULAR BIOLOGY OF FOOD ANIMAL GROWTH, DISEASE RESISTANCE, AND PREVENTION

BIRD R C; STRINGFELLOW D; Microbiology; Auburn University, Auburn, **ALABAMA** 36830.
Proj. No.: ALAV-0162 Project Type: STATE
Agency ID: CSVM Period: 01 OCT 84 to 30 SEP 89

Objectives: Define and isolate genes which perform a regulatory function in the cell cycle. Utilize recombinant DNA technology to study disease resistance and prevention.

Approach: Recombinant DNA technology will be used to locate regulatory genes expressed during the late G1 periods of the cell cycle and/or attempting to functionally compliment known cell cycle mutants in yeast with fragments of mammalian DNA. Subsequent work will focus on the characterization of these genes by restriction endonuclease mapping, DNA sequencing, in vitro mutagenesis and reinsertion of the mammalian genome in tissue culture cells. These and similar approachs will be used to study disease resistance and methods of disease prevention.

Progress: 87/10 to 88/09. Genetic Regulation of Cell Growth: Mammalian cells have been synchronized into seven sequential cell cycle phases by contrifugal elutriation. Cell cycle phase of each fraction has been determined by thymidine incorporation pattern over six sequential, one hour periods following elutriation and by measurement of histone H4 C-fos, and alpha-actin mRNA levels in cells from each fraction. Two cDNA populations (one to G1 and one to S phase specific mRNAs) are being prepared to enable cloning of a high Rotfraction, subtractive G.-specific cDNA Library. Ovine Uterine-Derived Growth Factor (O-UDGF) A very potent growth factor activity has been identified in uterine secretions collected from unilaterally pregnant sheep, by analysis in a bio-assay system based upon quiescent 3T3 fibroblasts. Mitogenic activity in excess of that observed in fetal bovine serum or adult sheep plasma was measured (per mg protein) and was shown to be relatively heat stable, though boiling would inactivate it, and greater than 30 KJ in native size. O-UDGF would specifically mitogenically stimulate both ovine trophoblasts as well as myoblasts above that observed in other cell lines.

Publications: 87/10 to 88/09

BIRD, R.C., NUSBAUM, K.E., SCREWS, E.A., YOUNG-WHITE, R.R., GRIZZLE, J.M. and TOIVIO-KINNUCAN, M. 1988. Molecular Cloning of Fragments of the Channel Catfish Virus (Herpesviridae) Genome and Exp. of the Encoded mRNAs During Infection.

NEWTON, J., BIRD, R.C., BLEVINS, W. and WOLFE, L. 1988. Isolation, Characterization and Molecular Cloning of Cryptic Plasmids Isolated From Virulent Edwardsiella ictaluri. Am. J. Vet. Res., 49:1856-1860.

BIRD, R.C., BARTOL, F.F., DARON, H., STRINGFELLOW, D.A. and RIDDELL, G.M. 1988. Mitogenic Activity in Ovine Uterine Fluids: Characterization of a Growth Factor Which Specifically Stimulates Myoblast Proliferation.

30.002 CRIS0138386
EXPRESSION OF BOVINE HERPESVIRUS-4 GENOME IN TISSUE CULTURE

VAN SANTEN V; Pathobiology; Auburn University, Auburn, **ALABAMA** 36830.
Proj. No.: ALAV-0202 Project Type: STATE
Agency ID: CSVM Period: 01 JAN 89 to 30 SEP 91

Objectives: Establish Bovine Herpesvirus-4 (BHV-4) recombinant DNA clones and generate physical restriction endonuclease map. Characterize RNA transcribed from BHV-4 genome during latent, non-productive, and different stages of productive infection in tissue culture. Determine whether BHV-4 infection stimulates host cell DNA synthesis, growth transformation, or bovine leukemia virus (BLV) transcription.

Approach: Pst1 and Hind3 restriction enzyme fragments representing the entire genome of BHV-4 American prototype strain DN-599 will be cloned. RNA transcribed from the BHV-4 genome during different stages of infection in tissue culture will be characterized using the cloned restriction fragments by Northern blot analysis, S1 nuclease analysis, and hybridization of radiolabeled cDNA. Growth tranformation experiments will use primary bovine embryo cells, bovine lymphocytes, and mouse 3T3 cells, and intact BHV-4 virions as well as viral DNA fragments. Stimulation of BLV transcription will be tested by infecting cells containing a BLV-LTR-chloramphenicol acetyl transferase reporter gene plasmid.

30.003 CRIS0099973
MOLECULAR & CELLULAR MECHANISMS OF BOVINE DEVELOPMENT

YOUNG R B; Office of Research Admin.; University of Alabama, Huntsville, **ALABAMA** 35899.
Proj. No.: ALAR-8603029 Project Type: CRGO
Agency ID: CRGO Period: 01 AUG 86 to 31 JUL 88

Objectives: Project 8603029. The primary objectives are to understand the structure and function of the bovine myosin heavy chain genes using recombinant DNA techniques.

Approach: RNA isolated from fetal, neonatal and adult bovine white skeletal muscle red skeletal muscle, cardiac muscle, smooth muscle, brain and liver will be used for the preparation of northern blots for hybridization against each of the clones. Coarse restriction maps will be constructed following digestion with a series of restriction enzymes, and the orientation of transcription will be determined. Subcloning and detailed analysis if selected regions of 3′ ends of myosin clones for subsequent use as specific probes for expression of bovine isoforms will be carried out. DNA sequence analysis of subclones in the 3′ coding and noncoding regions will be carried out, and evolutionary diversity will be studied.

30.004 CRIS0130201
MOLECULAR BIOLOGY OF MALIGNANT CATARRHAL FEVER VIRUSES

HEUSCHELE W; Zoological Society - San Diego; San Diego Zoo, San Diego, **CALIFORNIA** 92112.
Proj. No.: CALR-8601260 Project Type: CRGO
Agency ID: CRGO Period: 15 AUG 86 to 31 JAN 89

Objectives: PROJ 8601260. To clone the MCF viral genome (WC-11 strain) and generate a restriction enzyme cleavage map for comparison with other MCF virus isolates and other herpeviruses. Year I (1986-87). Preparation of molecular DNA probes specific for detection of MCF viral DNA in tissue samples from suspected viral carriers or those showing acute clinical disease. Year II (1987-88). To compare the relatedness of MCF virus isolates by analysis of purified MCF viral DNA with restriction endonuclease patterns. Comparisons of field isolates with known MCF strains (e.g., WC-11, c500 prototypes) as well as with each other will be done in this manner. Year II (1987-88).

Approach: Established molecular biologic methods for herpesviral DNA isolation, R.E. analysis and cloning will be used with cell culture propagated MCF virus.

Progress: 88/01 to 88/12. Alcelaphine herpesviruses (AHV) isolated from wildebeest replicate in both fetal aoudad sheep kidney (FAK) cells and bovine embryonic lung (BEL) cells. However, virus isolates from topi and hartebeest replicate only in FAK cells. Buoyant density analysis by analytical ultracentrifugation, restriction endonuclease analysis and blot hybridization of virus genomic DNA from both sets of alcelaphine herpesviruses as well as from bovine herpesviruses 1, 2, and 4 demonstrate that there are two types of alcelaphine herpesviruses, each distinct from the other bovine herpesviruses. Genomic size of both alcelaphine herpesviruses estimated from DNA restriction fragments is approximately 110 kilobase pairs. Alcelaphine herpesvirus DNA resembles Herpesvirus saimiri DNA during equilibrium sedimentation in that while the majority of the DNA bands as a light (L) fraction there is also a minor heavy (H) component. Analysis of virion proteins indicates that each alcelaphine herpesvirus has 31 polypeptides ranging in molecular weight from 12,000 to 275,000 but that polypeptides from each virus produce a distinctive pattern when separated by polyacrylamide gel electrophoresis. Virus isolates from wildebeest have been designated AHV-1 while viruses isolated from topi and hartebeest have been designated AHV-2.

Publications: 88/01 to 88/12

SEAL, B.S., KLIEFORTH, R.B., CASTRO, A.E. and HEUSCHELE, W.P. Replication of alcelaphine herpesviruses in various cell culture systems and subsequent purification of virus. J. Tissue Culture Methods 11:49-56. 1988.

WAN, S.K., CASTRO, A.E., HEUSCHELE, W.P. and RAMSAY, E.C. Enzyme-linked immunosorbent assay for the detection of antibodies to the alcelaphine herpesvirus of malignant catarrhal fever in exotic ruminants. Am. J. Vet. Res. 49:164-168, 1988.

NIELSEN, N.O., OOSTERHUIS, J., JANSSEN, D., MCCOOL, K., ANDERSON, M.P. and HEUSCHELE, W.P. Fatal respiratory disease in Nilgiri tahr: Possible malignant catarrhal fever. Can. J. Vet. Res. 52:216-221, 1988.

SEAL, B.S. and ST. JEOR, S.C. Purification and characterization of bovine herpesvirus-1 isolates and virus DNA utilizing bovine embryonic lung cells. J. Tissue Culture Methods 11:57-64, 1988.

SEAL, B.S., HEUSCHELE, W.P. and KLIEFORTH, R.B. Prevalence of antibodies to alcelaphine herpesvirus-1 and nucleic acid hybridization analysis of viruses isolated from captive exotic ruminants with malignant catarrhal fever.

HEUSCHELE, W.P., SEAL, B.S. and KLIEFORTH, R.B. Malignant catarrhal fever in wild ruminants. Verh. ber. Erkrg. Zootiere. 30:45-55, 1988.

SEAL, B.S., KLIEFORTH, R.B. and HEUSCHELE, W.P. Alcelaphine herpesviruses one and two: PAGE analysis of virus polypeptides, restriction endonuclease analysis.

30.005 CRIS0097072
MORAXELLA BOVIS PILI. MOLECULAR AND GENETIC STUDIES

SCHOOLNIK G K; FALKOW S; Medical Microbiology; Stanford University, Stanford, **CALIFORNIA** 94305.
Proj. No.: CALR-8502912 Project Type: CRGO
Agency ID: CRGO Period: 15 AUG 85 to 14 AUG 87

Objectives: Proj 8502912. The objectives are to: clone and sequence M. bovis pilin genes, determine functional and immunologic characteristics of pili, and prepare and test synthetic pilus peptide vaccine candidates.

Approach: M. bovis pilin genes will be cloned and sequenced using an oligonucleotide probe and standard molecular techniques. Pilus fragments and synthetic peptides will be used to elicit polyclonal antisera. Pilus characteristics will then be determined by combining these reagents in immune and adherence assays. Peptides which elicit cross reactive, adherence blocking antibodies will be used in prototype vaccines.

30.006* CRIS0138576
IMMUNOLOGY AND MOLECULAR BIOLOGY OF ECTOPARASITIC MITES

BOYCE W M; Veterinary Microbiology Immuno; University of California, Davis, **CALIFORNIA** 95616.
Proj. No.: CA-V*-VMB-5093-H Project Type: HATCH - PENDING
Agency ID: CSRS Period: 11 JUL 89 to 30 SEP 93

Objectives: To develop an immunoassay for diagnosis of mite infestations, determine the genetic relatedness of mites using molecular analysis of parasite DNA; and conduct epidemiologic studies of host specificity and treatment strategies.

Approach: Immunoassays will be developed using antigens extracted from mites and sera from naturally and experimentally infected animals. Cross transmission studies will be performed by transferring mites onto new hosts under experimental conditions. Genetic relatedness will be determined through a variety of molecular biology techniques including RFLP and DNA hybridization.

30.007 CRIS0134578
MOLECULAR CLONING OF THE GENOME OF THE ALCELAPHINE HERPESVIRUS I OF MALIGNANT CATARRHAL FEVER

ZEE Y; Microbiology & Immunology; University of California (vet-med), Davis, **CALIFORNIA** 95616.
Proj. No.: CALV-AH-104
Project Type: ANIMAL HEALTH
Agency ID: CSRS Period: 03 JUN 88 to 19 APR 93

Objectives: Restriction endonuclease mapping of field herpesviral isolates of malignant catarrhal fever (MCF) and comparison with prototype strains of MCF (e.g., WC11) to define differences and determine homology between viral strains. Molecular cloning of a representative herpesviral strain of MCF (WC11) to enable the development of a genetic probe which will recognize sequence homologies common for viral isolates of MCF.

Approach: The information obtained from this study will enable the development of a diagnostic probe which will recognize DNA sequences in tissue samples, leukocytes, or plasma from cattle or sheep suspected of harboring a latent infection with MCF or with clinical signs of an infection typical of MCF.

Progress: 88/01 to 88/12. A genomic probe specific for malignant catarrhal fever (MCF) virus was cloned by using purified viral DNA from MCF-virus strain WC11. Restriction endonuclease analysis of the purified viral DNA was used to identify the cloned viral genomic fragment. Dot blot hybridization by use of the genomic probe (pRP-5) indicated that the probe hybridized specifically with WC11-MCF virus, as well as with one other isolate of MCF-associated herpesvirus. Hybridization also was observed to a non-MCF virus strain of bovine herpesvirus.

Publications: 88/01 to 88/12
SHIH, L.; IRVING, J.M.; ZEE, Y.C.; PRITCHETT, R.F. (1988). Cloning and characterization of a genomic probe for malignant catarrhal fever virus. American Veterinary Medical Association; Vol. 49; No. 10; Pages 1665-1668.

30.008 CRIS0133517
AN INFECTIOUS VACCINIA VIRUS RECOMBINANT VACCINE FOR RINDERPEST

YILMA T; Veterinary Microbiology Immuno; University of California (vet-med), Davis, **CALIFORNIA** 95616.
Proj. No.: CALV-AID-86-17 Project Type: STATE
Agency ID: CSVM Period: 01 NOV 87 to 31 OCT 92

Objectives: To develop an effective infectious vaccinia virus recombinant vaccine to eradicate rinderpest.

Approach: The entire coding sequences of the two immunogenic genes (F and HA) of rinderpest virus will be cloned and then expressed in a single vaccinia virus recombinant. The immune response of cattle to the vaccine will be assessed by humoral immune response and be challenged with a virulent strain of rinderpest virus.

Progress: 88/01 to 88/12. Eight proteins of the rinderpest virus have been characterized; the HA gene has been sequenced and cloned; the F gene has been sequenced and cloned; infectious vaccinia virus recombinants have been prepared with the HA and the F genes; the recombinants have been used to vaccinate cattle, and have been shown to protect against greater than 1000x the normally-lethal dose of the virus.

Publications: 88/01 to 88/12
RUBMAN, M.; C. MEBUS; B. DALE; M. YAMANAKA; T. YILMA. (1988). Analysis of the polypeptides synthesized in rinderpest virus-infected cells. Virology 163:261-267.
HSU, D., M. YAMANAKA, J. MILLER, B. DALE, M. GRUBMAN, and T. YILMA. (1988). Cloning of the fusion gene of rinderpest virus: comparative sequence analysis with other morbilliviruses. Virology 166:251-253.
YAMANAKA, M., D. HSU, B. DALE, T. CRISP, M. GRUBMAN, and T. YILMA. (1988). Cloning and sequence analysis of the hemagglutinin gene of the virulent strain of rinderpest virus. Virology 166:251-253.
YILMA, T., D. HSU, L. JONES, S. OWENS, M. GRUBMAN, C. MEBUS, M. YAMANAKA and B. DALE. (1988). Protection of cattle against rinderpest with infectious vaccinia virus recombinants expressing the HA or F gene. Science 242:1058-1061.
YILMA, T., D. HSU, L. JONES, S. OWENS, M. GRUBMAN, C. MEBUS, M. YAMANAKA, and B. DALE. 1989. Expression of rinderpest genes in vaccinia virus Recombinants: Protective immunization of cattle. In: Chanock RM, Giensburg H, Lerner R, Brown F.

30.009 CRIS0098884
GENETIC ANALYSIS OF A PROTEIN KINASE STIMULATING PROTEIN SYNTHESIS

TRAUGH J A; DUGAICZYK A; Biochemistry; University of California, Riverside, **CALIFORNIA** 92521.
Proj. No.: CA-R*-BCH-4711-CG Project Type: CRGO
Agency ID: CRGO Period: 01 JUN 86 to 31 MAY 89

Objectives: Proj 8600072. Recently, we identified a protein kinase (PAK II) which is activated in response to growth promoting compounds and stimulates protein synthesis by phosphorylation of 40S ribosomal protein S6. Understanding this protein kinase at the molecular level is crucial to regulating the growth processes.

Approach: Thus we propose to: isolate pure PAK II from beef liver by ion-exchange chromatography and affinity chromatography utilizing established procedures and methodology; obtain partial primary sequences of purified PAK II and prepare synthetic DNA probes. Ultimately, we will identify the gene(s) for PAK II in cow, determine the primary sequence, and examine the effects of growth promoting compounds on transcription of PAK II mRNA.

30.010 CRIS0093997
POXVIRUS AS A CLONING VECTOR FOR IMMUNIZATION AGAINST BOVINE HERPESVIRUS 1

BLAIR C D; PEARSON L D; College of Vet Medicine; Colorado State University, Fort Collins, **COLORADO** 80523.
Proj. No.: COLV2004 Project Type: SPECIAL GRANT Agency ID: CSRS Period: 01 AUG 84 to 31 JUL 86

Objectives: Determine physical map location of glycoprotein genes on bovine herpesvirus 1 (BHV-1) DNA. Obtain molecular clones. Construct plasmids containing vaccinia virus promotor and non-essential DNA sequences. Insert clones BHV-1 DNA sequences into cloned vaccinia DNA sequences. Construct recombinant vaccinia virus mutants with incorporated BHV-1 genes. Determine immune response of laboratory animals and cattle to recombinant virus.

Approach: Molecular Clone BHV DNA fragments in expression plasmid. Select those whose products react with anti-glycoprotein monoclonal antibodies. Molecular clone vaccinia virus DNA fragments in pBR325. Construct recombinant plasmids by recombinant DNA methods. Simultaneously infect cultured cells with vaccinia virus and transfect with recombinant plasmids. Vaccinate mice with recombinant virus. Measure cell mediated immunity by Cr release.

Progress: 86/01 to 86/07. A library of monoclonal antibodies (MAb) was produced which denatured glycoproteins of bovine herpesvirus type 1 (BHV-1). Two MAb reacted with a 90,000/180,000 dalton protein complex and 10 MAb reacted with a 55,000/70,000/120,000 dalton protein complex. MAb which reacted with the 90K/180K complex partially competed with each other in protein binding assays, and neither neutralized virus infectivity. MAb which reacted with the 55K/70K/120K complex competed completely with each other in binding to proteins, but only 7 of them neutralized virus. The MAb have been used to map genes which code for glycoproteins as follows. Fragments of BHV-1 DNA with a size range of 500-1500 bp were produced by shearing. These fragments were inserted into the DNA of the expression vector lambda gt-11. The recombinant phage were screened by blotting plaques onto nitrocellulose and assaying for reactivity with MAb. Molecular hybridization showed that phage which produced a protein that was reactive with MAb to the 55K/70K/120K dalton complex contained a DNA insert from the Hind III A restriction fragment of BHV-1 DNA. More detailed mapping is in progress. A virus variant was isolated from BHV-1 stocks which infects mice. Further characterization of this variant is in progress.

Publications: 86/01 to 86/07

MCGRANE, V. 1985. Characterization of bovine herpesvirus 1 envelope glycoproteins with monoclonal antibodies. M.S. Thesis, Colorado State University, 114 p.

BLAIR, C.D., PEARSON, L.D., DUNN, D.C. and CHAPLIN, K.L. 1985. The mouse as a model for bovine herpesvirus-1 infections. Am. Soc. Virology Annual Meeting, Albuquerque, N.M., p. 41.

PEARSON, L.D., BLAIR, C.D., and CHAPLIN, K.L. 1985. Infection of BALB/C mice with bovine herpesvirus 1. Rocky Mountain Immunologists Abstracts, Aspen, CO, p. 10.

BLAIR, C.D., MCGRANE, V., TERAMOTO, Y. and MUIRHEAD, J. 1986. Monoclonal antibodies to denatured bovine herpesvirus-1 glycoproteins. Am. Soc. Virology Annual Meeting, Santa Barbara, CA, p. 23.

30.011 CRIS0098375
DEVELOPMENT OF A RECOMBINANT DNA DERIVED VACCINE AGAINST BOVINE ANAPLASMOSIS

BARBET A F; College of Vet Medicine; University of Florida, Gainesville, **FLORIDA** 32611.
Proj. No.: FLA-VME-02553 Project Type: CRGO Agency ID: CRGO Period: 01 APR 86 to 31 AUG 88

Objectives: 1) Characterize recombinant clones of E. coli expressing epitopes of AM 105; 2) obtain the sequence of the gene coding for Am 105; 3) localize Am 105 epitopes which induce neutralizing monoclonal antibodies.

Approach: The approach is to use recombinant DNA technology, including the use of monoclonal antibodies, to accomplish the objectives.

Progress: 87/10 to 88/09. A surface protein complex from Anaplasma marginale consisting of two polypeptides (of approximately 105,000 molecular weight in a Florida isolate) provides protection against challenge with virulent organisms. The genes coding for complete recombinant analogues of each polypeptide have now been cloned, partially sequenced and a promoter region identified. Analysis of one of these genes, coding for an epitope recognized by neutralizing monoclonal antibody, reveals a tandemly repeated sequence specifying a 29 amino acid repeating peptide. Two neutralizing monoclonal antibodies recognize synthetic peptides composed of this sequence in ELISA assays. Cattle sera from animals immunized with the native protein complex and protected

against challenge also react strongly with this peptide. The synthetic peptide can be polymerized without losing reactivity with neutralizing antibodies. Current experiments center on defining ways to present either the synthetic peptide or complete recombinant polypeptides to cattle to stimulate protective immunity.

Publications: 87/10 to 88/09

BARBET, A.F., PALMER, G.H., MYLER, P.J., MCGUIRE, T.C.: Characterization of an immunoprotective protein complex of Anaplasma marginale by cloning and expression of the gene coding for poly-peptide Am105L. Infection & Immunity 55:2428-2435.

OBERLE, S.M., PALMER, G.H., BARBET, A.F., MCGUIRE, T.C.: Molecular size variations in an immuno-protective protein complex among isolates of Anaplasma marginale. Infection and Immunity 56:1567-1573, 1988.

PALMER, G.H., OBERLE, S.M., BARBET, A.F., GOFF, W.L., DAVIS, W.C., MCGUIRE, T.C.: Immunization of cattle with a 36-kilodalton surface protein induces protection against homologous and heterologous Anaplasma marginale challenge.

30.012 CRIS0136061

RANGE OF HYPOBIOTIC STRAINS OF OSTERTAGIA OSTERTAGI ASSESSED USING DNA MARKERS

DAME J B; COURTNEY C H; Horticulture & Landscape Architecture; University of Florida, Gainesville, **FLORIDA** 32611.

Proj. No.: FLA-VME-02768 Project Type: SPECIAL GRANT
Agency ID: CSRS Period: 01 SEP 88 to 31 AUG 91

Objectives: The primary objectives of this project are to: (1) identify DNA markers which are capable of distinguishing strains of Ostertagia ostertagi with the capacity to undergo hypobiosis (arrested larval development) during the summer months from strains which arrest their development during winter, and (2) use these markers to determine the prevalence and geographic range of these strains in an area where their distribution overlaps (Virginia).

Approach: Clone multi-copy genes and repetitive DNA sequences for probes to differentiate Ostertagia ostertagi strains by DNA hybridization analysis. Use DNA probes to analyze samples of helminth eggs obtained from cattle from known farms of origin in Virginia.

30.013* CRIS0136409

SEX-SPECIFIC DNA IN LIVESTOCK ANIMALS

MCGRAW R A; College of Vet Medicine; University of Georgia, Athens, **GEORGIA** 30602.

Proj. No.: GEOV-0186 Project Type: STATE
Agency ID: CSVM Period: 01 JUL 87 to 30 JUN 91

Objectives: This project is aimed at identifying and characterizing sex-specific DNAs in economically important livestock species. The basic genetic information can be used to develop sex-specific DNA probes with potential application in assays for sex-fractionation of semen and/or sex determination of embryos.

Approach: The approach is to compare DNAs derived from male and female animals of each species by a variety of molecular genetic methods, including restriction analysis, cloning, sequencing, and hybridization techniques. DNA sequences unique to one of the sexes are then characterized and developed as sex-specific hybridization probes.

Progress: 87/07 to 88/12. This research is aimed at identifying and characterizing sex-specific DNAs in economically important livestock species. The genetic information is used to develop sex-specific DNA probes with potential application in assays for sex-fractionation of semen and in sex-identification of embryos. Methods include a variety of DNA manipulations: restriction enzyme digestions, electrophoretic separations, construction and propagation of recombinant DNA in bacteria, DNA sequece analysis, chemical DNA synthesis, enzymatic DNA amplification, and hybridizations using radioactively labelled probes. At this time, we have developed sex-specific probes in pigs and chickens. The procine probe has been used successfully for sex-identification of procine embryos and efforts are underway to attempt sex-fractionation of boar semen. Preliminary data suggests that we will be able to develop similar probes in horses and cattle.

Publications: 87/07 to 88/12

MCGRAW, R.A., JACOBSON, R.J. and AKAMATSU, M. 1988. A male-specific repeated DNA sequence in the domestic pig. Nucleic Acids Research 16(21):10389.

30.014 CRIS0134012

GENE SYNTHESIS AND EXPRESSION OF PROTEIN PRODUCTS FOR NATURAL AND MUTANT HEAT-STABLE ENTEROTOXIN

TRUMBLE W R; Bacteriology & Biochemistry; University of Idaho, Moscow, **IDAHO** 83843.

Proj. No.: IDA00911 Project Type: HATCH
Agency ID: CSRS Period: 01 JAN 88 to 31 DEC 92

Objectives: Constructing a synthetic gene and expressing active heat-stable enterotoxin (ST-1) and inactive mutant analog peptides. The synthetic gene(s) will be used to attempt to make the enterotoxin immunoreactive for vaccine development and to allow homologous recombination experiments to produce an infective E. coli incapable of producing active enterotoxin for use as a veterinary treatment to prevent enterotoxigenic EV coli diarrheal disease.

Approach: The published amino acid sequence of the ST-1 peptide has been back translated into nucleotide sequence. Several important design

features have been incorporated into the gene design. Synthetic oligonucleotides will be constructed containing mutations for specific amino acids and after combining the oligonucleotides to produce double strand ST-1 genes, DNA probes will be used to isolate genes encoding native and mutant ST-1 peptides.

Progress: 88/01 to 88/12. The E. coli toxin, STIa, causing infant and traveler's diarrhea in humans and collibacilosis in cattle, sheep and pigs, is an 18 amino acid peptide which is too small to be recognized by the immune system. At present, there is no effective vaccine or prophylactic treatment against STIa-mediated diarrheal disease. We are using two approaches to provide protection against the effects of this toxin. First, a synthetic gene has been constructed which links repeating units (encoding 17 of the 18 amino acids) of the STIa gene to produce a "multimeric" gene capable of expressing a large, antigenic protein which should have multiple epitopes in common with the native toxin. We have cloned "multimeric genes" which encode from two to ten linked-copies of the monomeric "core" sequence. The recombinant proteins will be tested for immune response, toxicity and neutralizing ability. Second, we have constructed a synthetic analog of the toxin gene. Putatively important amino acids have been deliberately modified through changes at the DNA level to produce a non-toxic STIa-analog protein. The "non-functional" gene-encoded protein will be compared to activity of a synthetic "wildtype" gene we have constructed. Using insertional sequences, we will attempt to insert this non-functional gene in place of the active toxin-producing gene to construct a biological control organism which would be capable of colonizing the gut but would be incapable of producing an active STIa-toxin.

Publications: 88/01 to 88/12
No publications reported this period.

30.015 CRIS0132264
DETECTION OF MAJOR GENES FOR GROWTH USING GENETIC MARKERS

LEWIN H A; Animal Science; 1301 West Gregory Drive, Urbana, **ILLINOIS** 61801.
Proj. No.: ILLU-35-0508 Project Type: CRGO
Agency ID: CRGO Period: 01 JUN 87 to 31 MAY 90

Objectives: We propose to use a genotypic and phenotypic evaluation of a large paternal sibship to identify genes with a major effect on growth and development. The specific aims of this grant are the following: Identification of genes associated with abnormal prenatal development and/or sperm maturation by determining segregation ratios for each of six paternal genetic marker systems; evaluation of BoLA and red blood cell antigen locus haplotypes for their effect on birth weight, weaning weight and yearling weight; evaluation of BoLA and red blood cell antigen locus haplotypes for their effect on carcass weight, fat thickness and ribeye area. PROJ. 8700165.

Approach: Half-sib bull calves will be selected for breeding studies based on marker haplotype(s) with positive and negative effects on growth or carcass traits. These breeding studies will allow us to determine whether genetic markers can be used for within sire selection programs and will provide a rationale for genetic screening at the population level.

Progress: 87/10 to 88/09. Parental half-sibs were used to detect associations between genetic markers and quantitative traits in beef cattle. A half-sib family (n = 146) was selected whose sire was known to be heterozygous at six polymorphic loci; BoLA-A (class I major histocompatibility complex), B, C and F (red blood cell systems), transferrin and vitamin D binding protein. Segregation of alleles fit the expected 1:1 Mendelian ratios for the RBC-B and F, Tf and BoLA-A loci. There was apparent segregation distortion in the RBC-C and Gc systems, which was due to the large number of uninformative matings at these loci. Least squares means were compared for differences in performance and carcass traits (steers only, n = 61) between groups of half-sibs that inherited alternate paternal alleles. Significant effects were found for two of the six marker systems. Half-sibs that inherited the chromosomal segment (CS) marked by the RBC-B system BGKO(subscript x)Y(subscript 2)A'O' phenogroup had heavier 205-day (9.1 kg) and 365-day (17.3 kg) adjusted weights, faster pre-weaning average daily gains (.04 kg) and less fat thickness (-2.6 mm) than sibs that inherited the CS marked by I(subscript 2)Y(subscript 2)E'(subscript 1)Y'. Also, sibs that inherited the CS marked by the BoLA-w28 allele had larger rib-eye areas (4.1 cm) than sibs that inherited BoLA-w2. These data indicate the probable presence of QTL linked to the RBC-B and BoLA systems that affect pre-weaning growth and lean muscle content, respectively.

Publications: 87/10 to 88/09
BEEVER, J.E., J. A. STEWART, H. A. LEWIN and P. D. GEORGE. (1987). Genetic markers for detecting major genes for quantitative traits: segregation of BoLA haplotypes in paternal half sibs. International Symposium on the Molecular Biology.
BEEVER, J. E., P. D. GEORGE, R. L. FERNANDO, C. J. STORMONT and H. A. LEWIN. (1989). Genes affecting growth and carcass traits detected in a paternal half sib family using genetic markers. Animal Genetics (in press).
BEEVER, J. E., P. D. GEORGE, R. L. FERNANDO, and H. A. LEWIN. (1988). Genes affecting growth and carcass traits detected in a paternal half sib family using genetic markers. (1988) University of Illinois Beef Cattle Research Report.

30.016 CRIS0137128
MOLECULAR CLONING AND SEQUENCING OF BOLA CLASS II GENES

LEWIN H A; Animal Science; 1301 West Gregory Drive, Urbana, **ILLINOIS** 61801.
Proj. No.: ILLU-35-0347 Project Type: HATCH
Agency ID: CSRS Period: 01 JAN 89 to 30 SEP 92

Objectives: The objectives of the proposed research are to: 1) construct a cDNA library from the BL-3 cell line (a cell line which expresses high levels of BoLA class II molecules), 2) identify and subclone all unique class II cDNAs (1.3-1.5 kb) in this cDNA library, 3) sequence unique clones (products of different loci and alleles) and compare sequences with other MEC class II sequences in the GenBank database, and 4) develop locus-specific and allele-specific probes for DNA-typing and mapping the class II region of cattle.

Approach: The cDNA library, cloned in lambda gt 10, will be screened by colony hybridization using human class II cDNA probes. Unique and allelic clones will be subcloned in pUC19 and sequenced in their entirety by the dideoxy chain terminating method. Sequence analysis will be performed using the DNASTAR computer software.

30.017 CRIS0133485
MOLECULAR ANALYSIS OF THE BOVINE MAJOR HISTOCOMPATIBILITY COMPLEX

LEWIN H A; SCHOOK L B; Animal Science; 1301 West Gregory Drive, Urbana, **ILLINOIS** 61801.
Proj. No.: ILLU-35-0509 Project Type: CRGO
Agency ID: CRGO Period: 01 SEP 87 to 31 AUG 89

Objectives: Proj. 8701469. The goal of the proposed research is to extend our knowledge of the structure, organization and polymorphism of the bovine major histocompatibility complex (MHC) using immunological and molecular techniques. The specific objectives of this proposal are: define alleles of BoLA class IIa pha-chain & beta-chain genes by Southern blot analysis; identify restriction fragment length polymorphisms (RFLPs) associated with the serologically-detected class 1 BoLA-A locus; and determine the level of BoLA polymorphism by analysis of BoLA haplotypes at the population level.

Approach: Laboratory methods to accomplish these objectives will include Southern blot analysis using heterologous cDNA probes, tissue typing for BoLA class 1 antigens, and computer-assisted cluster analysis of RFLPs and linkage analysis. Information gained from this study will improve our ability to identify genetically-superior cattle based on genotype and will provide a valuable tool for investigating the genetic basis of disease resistance/susceptibility in this economically important species.

Progress: 87/10 to 88/09. Genetic analyses of the complement factor B (Bf) and tumor necrosis factor (Alpha) (TNF-(Alpha)) loci were performed in family of BoLA-A (MHC class I) serotyped paternal half-siblings. Southern blot hybridizations of TaqI digested bovine genomic DNA to the murine cDNA TNF-a probe (pGem-3-cach) resulted in positive hybridization with 3.1 and 4.3 kilobase (kb) restriction fragments that were not polymorphic in the sample tested. Hybridization with a human cDNA Bf probe (pFB3b) revealed three positively hybridizing TaqI restriction fragments of 2.8, 3.2 and 4.5 kb. The 2.8 and 3.2 kb fragments were present in all individuals tested. The 4.5 kb fragment was polymorphic and was found to cosegregate in half-sibs with maternally-transmitted BoLA-w24 bearing haplotypes. The sire, being a BoLA-w2/w28 heterozygote, did not have the 4.5 kb allelic fragment, so that segregation was informative for each dam having the 4.5 kb allele. Interestingly, a 4.5 kb polymorphic TaqI restriction fragment, has also been described for human Bf, raising the possibility that this polymorphism predates the evolution of humans and cattle. Mapping of the Bf locus to the bovine MHC will facilitate our efforts to obtain a fine structure genetic map of the BoLA system.

Publications: 87/10 to 88/09

J. A. SHADDUCK, J. A. STEWART, D. I. WATKINS, M. E. STONE, H. A. LEWIN, and N. L. LETVIN. (1989). Comparison of serotyping, 1-D IEF and RFLP pattern analysis for detection of BoLA polymorphism. Animal Genet. (abstract in press).

LEWIN, H. A. (1989). Disease resistance and immune response genes in cattle: strategies for their detection and evidence of their existence. J. Dairy Sci. (in press).

TEUTSCH, M. R., L. B. SCHOOK, and H. A. LEWIN. (1989). Linkage of complement factor B gene to the bovine major histocompatibility complex. Fed. Proc, (abstract in press).

WATKINS, D. I., J. A. SHADDUCK, C. E. RUDD, M. E. STONE, H. A. LEWIN, and N. L. LETVIN. (1988). Isoelectric focusing of bovine major histocompatibility complex class II molecules: evidence for two expressed beta loci. (submitted).

WATKINS, D. I., J. A. SHADDUCK, M. E. STONE, H. A. LEWIN, and N. L. LETVIN. (1988). Isoelectric focusing ot bovine major histocompatibility complex class I molecules: evidence for a BoLA-B locus. (submitted).

30.018 CRIS0096793
STRUCTURE AND ORGANIZATION OF BOVINE IMMUNOGLOBULIN GENES

KNIGHT K L; Health Sciences Center; 809 South Wright Street, Champaign, **ILLINOIS** 61820.
Proj. No.: ILLR-8502208 Project Type: CRGO
Agency ID: CRGO Period: 01 SEP 85 to 31 AUG 87

Objectives: Proj. 8502208. The overall goal of this proposal is to characterize the bovine humoral immune system at the molecular

biological level.

Approach: Construct complete cosmid and phage bovine DNA recombinant libraries; isolate and characterize the genes encoding the immunoglobulin heavy chains, kappa and lambda chains, and secretory component; determine the nucleotide sequence of the cloned genes; determine the number of genes encoding each of the Ig polypeptides and search for restriction fragment length polymorphisms (RFLPs); express the bovine Ig genes in vitro by: a) cloning the Ig genes into eukaryotic expression vectors, b) transfecting these vectors into murine myeloma cells; and c) isolating and characterizing the expressed Ig polypeptide chains. Determine whether non-expressed kappa and lambda chain genes are rearranged in kappa and lambda expressing cells.

30.019 CRIS0131805
FACTORS INFLUENCING EMBRYO SURVIVAL IN DOMESTIC FARM ANIMAL SPECIES

FORD S P; Animal Science; Iowa State University, Ames, **IOWA** 50011.
Proj. No.: IOW02825 Project Type: HATCH
Agency ID: CSRS Period: 01 JUL 87 to 30 SEP 91

Objectives: Investigate the effect of SLA haplotype on the rate of development and survival of pig embryos, and develop plans to improve litter size by identifying SLA haplotypes of breeding pairs. Continue investigations on hormonal control of uterine blood flow throughout pregnancy in the ewe, cow and sow, with emphasis on the role of the uterine vasculature in conceptus growth and survival. In vitro perfusion of the bovine placentome as a "mini placenta" to study the factors which control the flow of blood to the fetal-maternal interface in the cow as well as factors which control bovine placental steroid production.

Approach: Identify SLA haplotypes in pigs by restriction fragment length polymorphism analysis. Relate these SLA haplotypes to developmental rate of embryos by: identification of SLA antigens on very early pig embryos utilizing an enzyme-linked immunosorbent assay (ELISA), counting the number of blastomeres of preimplantation pig embryos of different SLA haplotypes during defined periods during early pregnancy, and evaluation of the relative survival of embryos of different SLA haplotypes. Use chronic in vivo measurement of uterine blood flow (electromagnetic flow probes, microspheres, etc.), and in vitro perfusion of the bovine placentome, to investigate factors which control flow through the uterine and placental vasculatures throughout the periods of embryo and fetal development.

Progress: 88/01 to 88/12. Effect of PGF(subscript 2)(Alpha) on porcine corpora lutea (CL) following administration on day 9 of the estrous cycle. During the period prior to day 12 of the estrous cycle, porcine CL refractory to the luteolytic effects of PGF(subscript 2)(Alpha). We investigated functional and structural aspects of the effects of PGF(subscript 2)(Alpha) on porcine CL during the refractory period. Gilts were unilaterally ovariectomized on day 8 and utero-ovarian venous (UOV) and femoral arterial (FA) catheters were inserted. Gilts received 20 mg PGF(subscript 2)(Alpha) or vehicle on day 9 and the remaining ovary was removed on day 12. Progesterone declined markedly in the FA (3 hrs) and UOV (2 hrs) following PGF(subscript 2)(Alpha), but not vehicle, and had returned to pretreatment levels by day 11. Luteal growth (weight, protein and DNA content) continued in pigs from day 8 to day 12 and was not affected by a luteolytic dose of PGF(subscript 2)(Alpha) on day 9. These data suggest that PGF(subscript 2)(Alpha) administration on day 9 of the estrous cycle in pigs has transient inhibitory effects on luteal function without effects on luteal composition. Effect of intraluteal estradiol-17(Beta) implants on weight and progesterone secretion of porcine corpora lutea (CL). Estradiol-17(Beta) (E(subscript 2)) decreases the effectiveness of prostaglandin F(subscript 2)(Alpha) (PGF(subscript 2)(Alpha)) to induce luteolysis, and E(subscript 2) locally increases CL wt in pigs suggesting a direct luteotropic effect.

Publications: 88/01 to 88/12

GUENTHER, A.E., CONLEY, A.J., VAN ORDEN, D.E., FARLEY, D.B. and FORD, S.P. (1988). Changing structural and mechanical properties of uterine arteries during porcine gestation. J. Anim. Sci. 66:3144-3152.

FORD, S.P., SCHWARTZ, N.K., ROTHSCHILD, M.F., CONLEY, A.J. and WARNER, C.M. (1988). Influence of SLA haplotype on preimplantation embryonic cell number in miniature pigs. J. Reprod. Fert. 84:99-104.

LUND, J., FAUCHER, D.J., FORD, S.P., PORTER, J.C., WATERMAN, M.R. and MASON, J.I. (1988). Developmental expression of bovine adrenocortical steroid hydrolases: regulation of P-450(subscript 17)(Alpha) expression leads to episodic fetal cor.

CONLEY, A.J. and FORD, S.P. (1989). Effects of a phorbol ester (TPA), calcium ionophore (A23187) and prostaglandin F(subscript 2)(Alpha) (PGF(subscript 2)(Alpha)) on progesterone secretion by dispersed ovine luteal cells. Biol. Reprod.

CONLEY, A.J. and FORD, S.P. (1989). Direct luteotropic effect of oestradiol-17(Beta) on porcine corpora lutea. J. Reprod. Fert. (In press.).

CONLEY, A.J., PUSATERI, A.E. and FORD, S.P. (1989). Effects of prostaglandin F(subscript 2)(Alpha), (PGF(subscript 2)(Alpha)) on porcine corpora lutea (CL) following administration on day 9 or the estrous cycle. Proceedings Midwestern Sect.

30.020 CRIS0048466
PRODUCTION OF BRUCELLA SURFACE PROTEINS WITH RECOMBINANT DNA TECHNOLOGY

TABATABAI L B; MAYFIELD J E; Humanities & Science; Iowa State University, Ames, **IOWA** 50011.
Proj. No.: 3630-34000-003-01S
Project Type: COOPERATIVE AGREE.
Agency ID: ARS Period: 22 FEB 84 to 30 SEP 88

Objectives: Establish a clone library for the Brucella abortus genome and isolate clones that code for surface proteins. Subclone useful genes into E. coli plasmids to produce Brucella proteins and explore methods for purifying these proteins..

Approach: DNA extracted from B. abortus will be cleaved with restriction endonucleases, randomly cloned into lambda phage, and propagated on E. coli K12. The colone library will be screened for specific Brucella surface proteins. Brucella proteins expressed will be identified and characterized in comparison with similar products of direct extraction. E. coli plasmid-mediated synthesis of Brucella proteins will be evaluated and purification methods will be developed..

Progress: 88/01 to 88/12. Two new lambda clones have been identified which express 70kDa and 66kDa Brucella proteins, respectively. The antiserum (R213) used to identify these clones were raised against a small group of 60-80kDa proteins which are identified by cattle antisera from vaccinated and infected animals. A large amount of effort was also expended unsuc- cessfully in attempting to clone the gene coding for BCSP34. It seems likely that this protein is not expressed in the Brucella DNA library we are using. We completed a study to determine the cellular location of BCSP20, BCSP31, and BCSP45 in recombinant E. coli cells. All three proteins are located in the periplasm, and all three cause E. coli to nonspecifically leak periplasmic proteins into the growth medium. We have also constructed some of the subclones necessary to sequence BCSP20 and BCSP45. We have introduced plasmids coding for all three proteins into several strains of S. typhimurium. High expression of BCSP45 seems to be lethal, while BCSP20 and BCSP31 express as well as in E. coli. Preliminary data indicates that mice infected with S. typhimurium expressing BCSP31 produce circulating antibodies against this protein.

Publications: 88/01 to 88/12
MAYFIELD, J.E., BRICKER, B.J., GODFREY, H., CROSBY, R.M., KNIGHT, D.J., HALLING, S.M., BALINSKY, D. and TABATABAI, L.B. 1988. Cloning, expression, and nucleotide sequence of a gene cloning for an immunogenic Brucella protein. Gene 63:1-9.

30.021* CRIS0004941
CONSULTATION AND RESEARCH IN MATHEMATICAL AND STATISTICAL GENETICS

POLLAK E; Statistics; Iowa State University, Ames, **IOWA** 50011.
Proj. No.: IOW01448 Project Type: STATE
Agency ID: SAES Period: 01 JUL 59 to 01 JAN 99

Objectives: Study of population genetics, with particular reference to balanced polymorphisms maintained by natural selection occurring in human and other species. Consultation on mathematical problems arising from workers in genetics.

Approach: Procedure will consist partly of the examination of theoretical models and will be partly in cooperation with individuals who have collected or are collecting data on genetic populations.

Progress: 88/01 to 88/12. E. Pollak provided assistance to Dr. A. R. Hallauer of the Department of Agronomy, who asked a question concerning covariances between relatives when a population originally has a Hardy-Weinberg structure and successive generations are produced by self fertilization. Let FS(subscript n) be the mean of a full sib family resulting from a cross between two plants that are produced after n generations of selfing. It was verified that the covariance between FS(subscript 0) and FS(subscript 4) is equal to the covariance between full sib offspring of individuals of generation 0. Assistance was also provided to Mr. Brad Hedges, a student in the Department of Agronomy. He was faced with the problem of calculating what family size is large enough so that, if there are two possible sets of underlying frequencies of K types of offspring of a cross, the probabilities of the two kinds of misclassification are each 0.025. Professor C. P. Cox of the Department of Statistics and E. Pollak collaborated in solving this problem. Previously, the solution was known only if there are two types of offspring.

Publications: 88/01 to 88/12
JUNG, Y. C., ROTHSCHILD, M. F., FLANAGAN, M. P., POLLAK, E. and WARNER, C. M. Genetic variability between two breeds based on restriction fragment length polymorphisms (RFLPs) of major histocompatability complex class I genes in the pig.

30.022 CRIS0060069
BOVINE RESPIRATORY DISEASE, RISK FACTORS, PATHOGENS, DIAGNOSIS AND MANAGEMENT

ROSENBUSCH R F; ROTH J A; PAUL P S; Veterinary Medical Research Institute; Iowa State University, Ames, **IOWA** 50011.
Proj. No.: IOW01905 Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 86 to 30 SEP 91

Objectives: Antigens, immunogens, virulence factors and corresponding genomic sequences of the etiologic agents of bovine respiratory disease (BRD) will be identified. Specific and nonspecific resistance mechanisms which aid in

control and prevention of bovine respiratory disease will be defined and enhanced. The basic mechanisms of respiratory injury in BRD will be identified and related to the evolution of the pathophysiologic basis of the lesions. Techniques and practices related to diagnosis, prevention, treatment and management of BRD will be evaluated.

Approach: Specific antigens of Nycoplasma dispar and Hemophilus somnus will be characterized using monoclonal antibodies and physiochemical separation techniques. Genome fragments of M. dispar will be cloned to prepare expression libraries and hybridization probes to determine prevalence of M. dispar variants. Subpopulations of lymphocytes will be obtained from BVD-infected calves and examined for functional responsiveness to BRD agents. Characteristics of recombinant lymphokines will be studied. Immunomodulators will be studied in cattle vaccinated or infected with BRD agents or their antigens, and immunosuppression by BVD and BRSV will be assessed. Rapid detection of BVD will be attempted with monoclonal antibodies and DNA probes.

Progress: 88/01 to 88/12. Cytopathic, Oregon C24V, and noncytopathic New York 1 (NY1), strains of bovine virus diarrhea virus (BVDV) were studied in vitro, using bovine turbinate cells (BTU) and bovine endothelial cells (BEC). Virus behavior in the two cell types was compared using one-step virus growth curves, radioimmunoprecipitation (RIP), and cytotoxicity assays. Both C24V and NY1 replicated to overall higher titers in BEC's than in BTU's, but production of new virions appeared to occur in both cell types within the same time frame. In BEC's the NY1 virus synthesizes a polypeptide with an approximate molecular mass of 89-92 kD. This polypeptide is absent in NY1 infected BTU's. It has been shown previously (and confirmed in this work) that a 80-87 kD is synthesized by cytopathic BVD strains, while noncytopathic strains fail to produce this protein. In addition, transient cytoplasmic vacuolation could be observed in NY1 infected BEC's, but not in BTU's. In contrast, no cytotoxic activity induced by NY1 infection could be detected in either cell type using a 51-Chromium uptake assay. It was concluded that based on RIP data and morphological evaluations, viral polypeptide expression and cytopathology can be dependent on the host cell type.

Publications: 88/01 to 88/12
No publications reported this period.

30.023 CRIS0133008
JOHNE'S DISEASE: PRODUCTION OF ANTIGENS FOR USE IN DIAGNOSTIC TESTS

THOEN C O; Veterinary Microbiology & Preventive Medicine; Iowa State University, Ames, **IOWA** 50011.
Proj. No.: IOWV-416-23-07
Project Type: SPECIAL GRANT
Agency ID: CSRS Period: 01 JUL 87 to 30 JUN 91

Objectives: To develop a more specific diagnostic test without the undesirable cross reactions which interfere with the interpretation of diagnostic tests for paratuberculosis and to isolate these genes in E. coli. To prepare polyclonal antibodies against the same strain of M. paratuberculosis for screening the genomic library. To evaluate the proteins produced by selected genes in E. coli in a ELISA to detect specific antibodies in sera of M. paratuberculosis infected cattle.

Approach: DNA fragments of M. paratuberculosis will be cloned into plasmid pUC19. A genomic library will be prepared in E. coli using this plasmid. Mycobacterial proteins produced in E. coli will be evaluated by immunoblot and by ELISA using polyclonal antibodies obtained from cattle exposed to M. paratuberculosis.

Progress: 88/01 to 88/12. Genomic DNA was prepared from spheroplasts of M. paratuberculosis. The purified chromosomal DNA was partially digested with EcoRI and ligated into the EcoRI site of plasmid pUC19. The recombinant molecules were used to transform competent E. coli cells. The resulting transformants containing mycobacterial DNA inserts were screened by immunoblotting using serum from a bovine in which paratuberculosis was diagnosed. The molecular weights of immunogenic proteins were determined by analysis in a western blot. The sizes and physical maps of DNA segments encoding such proteins were compared.

Publications: 88/01 to 88/12
PIERCE, J.K., ANDREWS, R.E., THOEN, C.O. and WAITE, K. Cloning and Expression of Mycobacterium paratuberculosis Genes in Escherichia coli. Abstracts of 88th Annual Mtg. American Society for Microbiology, p. 132.

30.024 CRIS0133790
USE OF A HUMAN POLYMORPHIC DNA MARKER IN THE BOVINE SPECIES

TROYER D L; SMITH J E; LEIPOLD H W; Anatomy & Physiology; Kansas State University, Manhattan, **KANSAS** 66506.
Proj. No.: KAN081836Project Type: ANIMAL HEALTH
Agency ID: CSRS Period: 01 DEC 87 to 30 SEP 88

Objectives: To develop a synthetic oligonucleotide probe based on the core sequence of a tandemly repetitive region near the human gene for DNA fingerprinting and segregation analysis in the bovine genome.

Approach: Bovine genomic DNA will be extracted, purified, size fractionated on agarose gels, and transferred to nylon. A synthetic probe based on the insulin polymorphism will be labeled by the random primer technique and southern blot analysis will be utilized to analyze the bovine genome for similar polymorphisms.

Progress: 87/12 to 88/09. The human VNTR (variable number tandem repeat) probes PEKMDA, pJCZ 3.1, and PYNH24 were used in Southern blot

analysis of bovine DNA to ascertain their usefulness as polymorphic DNA markers in that species. The PUCZ31 probe, based on a region near the human zetaglobin psuedogene revealed a polymorphic pattern in unrelated bovine DNA digestd with the enzyme Ms PI. The other two probes, based on region in the hepatitis B virus genome revealed moderate polymporhisms within the bovine genome when the same restriction endonuclease was used. Although it was difficult to obtain excellent signal to noise ratios with these heterologous probes, they should be useful in gene mapping efforts in this species. In addition, the human marker PCMM was analyzed in the equine species and found to cross-hybridize with DNA from this species.

Publications: 87/12 to 88/09

TROYER, D., HOWARD, D, LEIPOLD, H.W. and SMITH, J.E. A human minisatellate sequence reveals DNA polymorphism in the equine species. Zentrabl Vet. Med. (in press).

30.025 CRIS0137865

A SEARCH FOR RESTRICTIVE FRAGMENT LENGTH POLYMORPHISMS (RFLP) IN THE BOVINE GENOME

SMITH J E; LEIPOLD H W; TROYER D; Pathology; Kansas State University, Manhattan, **KANSAS** 66506.

Proj. No.: KAN00794 Project Type: HATCH
Agency ID: CSRS Period: 01 JUL 89 to 30 SEP 92

Objectives: To locate RFLP's in the bovine genome for genetic markers of genetic diseases in cattle.

Approach: High molecular genomic DNA will be extracted from bovine leukocytes, digested with five-fold excess of various restriction endonucleases, and separated by agarose gel electrophoresis. The separated fragmented DNA will be transferred to nitrocellulose for Southern analysis using probes that reveal DNA polymorphisms in man.

30.026* CRIS0049916

CLONING DNA OF COCCIDIAN AND HELMINTH PARASITES FOR STRAIN IDENTIFICATION AND VACCINE PREPARATION

DAME J B; Biostematics Laboratory Animal Parasitology Institute; Beltsville Agr Res Center, Beltsville, **MARYLAND** 20705.

Proj. No.: 1265-34000-002-00D
Project Type: INHOUSE
Agency ID: ARS Period: 01 JUL 85 to 01 JUL 90

Objectives: 1) Develop a reliable system to distinguish between related strains and/or species of Trichinella, Ostertagia, Haemonchus and Eimeria based on differ-ences in the structure of their DNA; 2) clone and express in Escherichia coli, genes from Toxoplasma gondii and Eimeria spp., which encode antigens that may be useful in preparing vaccines or diagnostic tests for these parasites.

Approach: 1) Genomic DNA libraries will be prepared in plasmids of Escherichia coli and clones of repeated DNA sequences, ribosomal RNA genes and mitochondrialDNA identified. These clones will be used as DNA hybridization probes to detect strain and species differences by southern blot analysis. 2) Geno- mic DNA libraries will be prepared in the E. coli expression vector gambda gt11 by the mung bean nuclease method. Clones of genes encoding parasite antigens will be identified using antibodies. Recombinant-produced anti- gens will be compared with native antigens and their value as a vaccine component tested.

Progress: 88/01 to 88/12. Ribosomal DNA genes were cloned from Trichinella spiralis and subcloned in plasmid vectors for use as DNA probes. These subcloned fragments were useful in studying the systematics and in the characterization of T. spiralis (pig biotype) and T. pseudospiralis. A cloned DNA sequence was also identified from a T. spiralis cDNA expression library which has application in serodiagnosis. The characterization of these gene sequences provided information on the excretory-secretory antigens of this parasite. We isolated DNA from ethanol fixed cestode proglottids and determined genetic markers that support the recognition of a new species of Taenia from Taiwan. In an attempt to find an antigen for use in a vaccine against coccidiosis of chickens, DNA encoding a recombinant antigen of Eimeria acervulina merozoites was cloned after immunoscreening bacteriophage expression libraries. The purified recombinant antigen, believed to be a series of related surface proteins, stimulated T cells from Eimeria-immune chickens and when administered to susceptible chickens conferred partial protection. Sequencing of a cDNA encoding a portion of a second E. acervulina merozoite p250 surface protein revealed tandem-repeated DNA similar to structures reported for malaria. Similarities in protein structure between different protozoans of the Apicomplexa may imply similar means of evading immune responses by the host.

Publications: 88/01 to 88/12

ZARLENGA, D.S. and DAME, J.B. 1988. Molecular cloning and characterization of ribosomal RNA genes from Trichinella spiralis. The FASETS Journal 2(5):A 1028.

JENKINS, M.C. 1988. A cDNA encoding a merozoite surface protein of the protozoan Eimeria acervulina contains tandem-repeated sequences. Nucleic Acids Research. 16(20):9863.

JENKINS, M.C., DAME, J.B., LILLEHOJ, H.S., DANFORTH, H.D. and RUFF, M.D. 1988. Cloned genes coding for avian coccidiosis antigens. U.S. Patent Application, 7-155,264.

JENKINS, M.C., LILLEHOJ, H.S. and DAME, J.B. 1988. Eimeria acervulina: DNA cloning and characterization of recombinant sporozoite and merozoite antigens. Exp. Parasitol. 66:96-107.

LILLEHOJ, H.S., JENKINS, M. C., BACON, L.D., FETTERER, R.H. and BRILES, W. E. 1988. Protection against Eimeria acervulina correlates with T cell response to r ecombinant surface merozoite antigen. Exp.

Parasitol. 67:148-158.
JENKINS, M.C., LILLEHOJ, H.S., DANFORTH, H.D. and FETTERER, R.H. 1988. cDNA encoding antigens of Eimeria acervulina: DNA sequence analysis; T cell and B cell epitopes. Ann. Mtg. Fed. Exp. Biol., Las Vegas, NV (ABSTRACT).
JENKINS, M.C., STROHLEIN, D.A., DANFORTH, H.D. and LILLEHOJ, H.S. 1988. Cloning of genes encoding surface antigens of Eimeria acervulina sporozoites and merozoites. Ann. Mtg. Poult. Sci. Ass., Baton Rouge, LA (ABSTRACT).

30.027 CRIS0132149
MOLECULAR PROBES AS GENETIC MARKERS

PONCE DE LEON F A; Veterinary & Animal Science; University of Massachusetts, Amherst, **MASSACHUSETTS** 01003.
Proj. No.: MAS00628 Project Type: HATCH
Agency ID: CSRS Period: 01 JUL 87 to 30 SEP 92

Objectives: Prepare DNA libraries as sources of unique DNA sequences to be used as molecular probes. Characterization of isolated molecular probes. Initial screening for restriction fragment length polymorphisms (RFLPs). Mapping of RFLPs by in situ hybridization. Preparation and maintenance of the RFLP bank.

Approach: DNA libraries (genomic, cDNA and/or chromosome DNA libraries) will be screened for unique DNA sequences to be used as probes for the detection of Restriction Fragment Length Polymorphisms (RFLPs). Blood samples from at least 30 Holstein Fresian cows will be collected. Ficoll isolated leukocytes will be cultured after LPS stimulation. DNA extractions will be carried out from the cultured leukocytes by means of standard techniques (Davis et al., 1986), and stored at -20 C. Cultured leukocytes which are not destined for DNA extractions will be used for chromosome preparations.

Progress: 87/10 to 88/09. Work has been focused on: The preparation of approximately 100 DNA samples, from two different sources, aimed at providing a sample of unrelated individuals and of half sib families. This sample will provide the opportunity to detect restriction fragment length polymorphisms (RFLPs) and will allow us to define patterns of inheritance by differentiating RFLP alleles and homozygous from heterozygous individuals. An RFLP has been detected with the use of a genomic sequence of the bovine immunoglobulin gene (IgM). Characterization of the IgM RFLP is in progress. Establishment of a primary explant bovine fibroblast cell culture to be used as a source of bovine chromosomes for the preparation of cell hybrids (human X bovine) carrying single bovine chromosomes. This approach utilizes natural selectable markers and transfected selectable markers. The latter requires transfection of bovine fibroblast with a plasmid construct carrying a gene that confers resistance to the aminoglycoside antibiotic G418. The lethal dose f G418 to bovine fibroblasts and appropriate treatment to produce bovine micro-nuclei have been determined. Production of cell hybrids is under way. DNA microcloning: since chromosome specific DNA probes can be obtained by physical isolation of identifiable chromosomes from metaphase spreads, cloning of picogram quantities of DNA is necessary. Conditions for successful DNA micro-cloning have been worked out.

Publications: 87/10 to 88/09
SEITZ, A.W. and PONCE DE LEON, F.A. 1988. A simplified method for DNA microcloning. Annual Meeting of the American Society of Animal Science. Abstract. J. of Animal Science 66 (Supl. 1):223-224.

30.028 CRIS0099956
MOLECULAR STUDIES OF THE HEMOLYSIN GENE(S) OF MORAXELLA BOVIS

MARRS C; Epidemiology & Community Hlth; University of Michigan, Ann Arbor, **MICHIGAN** 48109.
Proj. No.: MICR-8601640 Project Type: CRGO
Agency ID: CRGO Period: 01 SEP 86 to 31 AUG 89

Objectives: PROJECT 8601640. The hemolysin gene(s) of Moraxella bovis will be cloned into E. coli, and the genes involved in hemolysin synthesis and transport will be determined. The structural gene for the hemolysin will be sequenced and used to develop highly expressed toxoid derivatives of hemolysin for use as potential vaccines.

Approach: Clones of M. bovis DNA in cosmid vectors in E. coli will be screened for acquisition of hemolytic ability, or indirectly obtained by first mutating the hemolysin gene by transposon mutagenesis and then cloning out the drug resistance. A highly expressing E. coli promoter will be fused to the structural gene and non-hemolytic, cross-reactive material producing mutants will be generated by site specific mutagenesis of the cloned gene(s).

Progress: 88/01 to 88/12. The goal of our project is to isolate and characterize the gene(s) involved in hemolysin production in Moraxella bovis. As reported last year, direct screening of 125,000 plaques from two lambda ZAP libraries of genomic M. bovis DNA for hemolytic plaques failed. Our attempt to label the hemolysin gene by transposon insertion mutagenesis has been stymied by an inability to get any plasmids into M. bovis by either conjugation or transformation. We are now trying electroporation, but so far we have been unable to electroporate plasmid DNA into M. bovis. We are still working on optimizing electroporation conditions reported in the literature for other bacteria and are hopeful that when we learn how to more efficiently electroporate other bacteria we will be able to have some success in M. bovis. Other approaches we are pursuing include: Dr. William Ruehl, our collaborator at Stanford, has isolated purified hemolysin as a band on an SDS-PAGE gel and has injected it into rabbits. When the resulting antibodies are isolated and confirmed to be directed against the hemolysin, we will use them as immunoprobes to screen the lambda ZAP

libraries as well as a lambda gt11 library constructed earlier. We are attempting to obtain a lambda library of Branhamella ovis, a closely related bacteria to M. bovis, which also produces a hemolysin. When the library is made we will directly screen it for hemolytic plaques.

Publications: 88/01 to 88/12
NO PUBLICATIONS REPORTED THIS PERIOD.

30.029 CRIS0130045
MOLECULAR CHARACTERISTICS OF BOVINE VIRAL DIARRHEA VIRUS

COLLETT M; Molecular Genetics; 10320 Bren Road East, Minnetonka, **MINNESOTA** 55343.
Proj. No.: MINR-8601071 Project Type: CRGO
Agency ID: CRGO Period: 15 SEP 86 to 14 SEP 87

Objectives: PROJECT 8601071. To characterize and sequence the 5′ and 3′ ends of the genomic RNA of bovine viral diarrhea virus (BVDV). To molecularly clone the BVDV (NADL isolate) genome. To DNA sequence the cloned genome. To express regions of the cloned genome as polypeptides in bacteria.

Approach: The genomic RNA of BVDV will be extracted from infected MDBK cells and purified by gel electrophoresis. This RNA will be radiolabeled with PpCp and T4 RNA ligase, and the 3′ end of the RNA will be sequenced. It will be determined if the 5′ end of this RNA is capped by subjecting metabolically P-labeled genomic RNA to enzymatic digestion, followed by two-dimensional thin-layer chromatography. Molecular cloning of the BVDV genome will employ standard cloning techniques using synthetic oligonucleotides as specific primers for reverse transcription. Resultant cDNA clones will be DNA sequenced by standard procedures. Open reading frames, as determined from the DNA sequence data, will be engineered into an E. coli expression plasmid so as to allow for the synthesis of BVDV specific polypeptides.

Progress: 86/09 to 87/09. Bovine viral diarrhea virus (BVDV) is an economically important pathogen of cattle throughout the world. The molecular characaterization of this virus has been limited. Our objectives for this one year grant were to molecularly clone and then sequence the genome of the NADL isolate of BVDV. Furthermore, we proposed to express, in E. coli plasmid-based systems, select regions of the cloned genome and use the resultant bacterially-produced BVDV analog polypeptides togenerate specific antisera. BVDV RNA was purified from infected cells by a combination of salt fractionation and gel electrophoresis. The RNA was then used for the synthesis of cDNA using reverse transcriptase. Second strand synthesis and insertion by homopolymeric tailing into a plasmid vector followed standard procedures. Plasmid inserts were determined to be BVDV-specific by colony and Northern format hybridization. cDNA inserts were characterized and aligned by restriction and cross-hybridization analyses. The DNA sequence of the BVDV inserts was determined for both strands Moreover, about 78% of the genome was sequenced from at least two independent cDNA clones. The sequence obtained was 12,573 nucleotides in length, which corresponds closely with the estimated size of the BVDV RNA. Analysis of the sequence revealed a single large open reading frame (ORF) in the positive (viral) polarity extending the length of the RNA. Assuming non-overlapping genes, the protein coding capacity of the ORF was 449 kilodaltons.

Publications: 86/09 to 87/09
NO PUBLICATIONS REPORTED THIS PERIOD.

30.030 CRIS0049435
IMMUNO- AND MOLECULAR GENETICS OF DISEASE RESISTANCE

STONE R T; Genetics & Breeding Research U.s. Meat Animal Research Ctr; Agricultural Research Service, Clay Center, **NEBRASKA** 68933.
Proj. No.: 5438-31000-002-00D
Project Type: INHOUSE
Agency ID: ARS Period: 01 OCT 84 to 30 SEP 89

Objectives: The objectives of this research are to define genetic polymorphisms, particularly in the major histocompatibility complex, and to relate the genotypes defined by these polymorphisms to disease resistance/ susceptibility in cattle.

Approach: Variants in the major histocompatibility complex will be defined immunologically with previously described antisera against lymphocyte antigens and by determining restriction fragment length polymorphism using homologous probes. Homologous recombinant DNA probes will be obtained by screening a bovine genomic library with heterologous probes andsubsequent subcloning.

Progress: 88/01 to 88/12. The structure and organization of the bovine major histocompatibility complex class II genes are being studied because of the role this gene family has in immune response and disease resistance. The nucleotide for the major exons of 2 DR beta-like genes and the B1 exon of a DQ beta-like gene has been obtained. The nucleotide sequence of the B1 exons of the DR beta-like genes (one is a pseudogene) are no more similar to each other than to the equivalent exons in other species, illustrating the well-known polymorphism in this region. The B2 exons are conserved in the two bovine genes and are about 90% identical to the equivalent in other species. The B1 exon of the DQ beta-like gene is similar in sequence in that of the human. These sequence data have helped to clarify some of the complex patterns observed when genomic blots of bovine DNA have been hybridized to MHC class II probes from other species. Using these bovine exon as probes, it has been possible to make allelic assignments to some of bands observed on genomic blots. The identification of particular DNA patterns or bands as alleles is very useful in assessing any statistical association between the MHC and disease/production traits.

Some class I MHC alleles show a statistical relationship to daily gain in cattle.

Publications: 88/01 to 88/12

STEAR, M.J., POKORNY, T.S., MUGGLI, N.E. and STONE, R.T. 1988. Breed differences in the distribution of BoLA-A locus antigens in American cattle. Anim. Genet. 19:171-176.
MUGGLI, N.E. and STONE, R.T. 1988. Identification of genetic variation in the bovine major histocomplex DR beta-like genes using sequenced bovine genomic probes. Anim. Genet. 19:213-225.
MUGGLI, N.E. and STONE, R.T. 1988. Characterization of a functional bovine DR beta-like gene and restriction fragment length polymorphisms associated with it. Proc. of XXIst Int. Conf. Anim. Bld. Grps. and Biochem. Polym. July 1988, Ital.
MUGGLI, N.E., STONE, R.T. and STEAR, M.J. 1988. Identification of a bovine DR beta-like pseudogene and the construction of probes for the definition of RFLPs of cattle. Anim. Genet. 19 (Suppl. 1):35-36.
MUGGLI, N.E., STEAR, M.J. and STONE, R.T. 1988. Investigations of the major histocompatibility complex in cattle and its association with economically important traits. Beef Res. Prog. Rpt. No. 3, USDA, ARS, ARM-NC-42, pp. 20-21.
STEAR, M.J., POKORNY, T.S., MUGGLI, N.E. and STONE, R.T. 1989. The relationship of birth weight, preweaning gain and postweaning gain with the bovine major histocompatibility system. (J. Anim. Sci. Accepted Aug. 24, 1988).

30.031 CRIS0133286

MOLECULAR ANALYSIS OF LATENT INFECTION WITH BOVINE HERPESVIRUS TYPE I

ROCK D L; Veterinary Science; University of Nebraska, Lincoln, **NEBRASKA** 68583.
Proj. No.: NEB-14-046 Project Type: SPECIAL GRANT
Agency ID: CSRS Period: 15 AUG 87 to 31 AUG 89

Objectives: The proposed specific objectives and approaches include: Identifying, with in situ hybridization, those regions of the BHV-1 genome that are transcriptionally active in neurons during the latent phase of infection and during the early stages of dexamethasone induced viral reactivation.

Approach: Identifying and mapping latency-related viral genes located within identified transcriptionally active regions using RNA hybrid selection/in vitro translation, RNA filter hybridization, and cross hybridization studies with herpes simplex virus type 1 (HSV-1).

Progress: 88/01 to 88/12. Transcription from the latency related (LR) region of BHV-1 (M.U. 0.734-0.748) was characterized in latently infected ganglia and lytically infected tissue culture cells. A LR-RNA of between 771 and 1169 bases was mapped in latently infected ganglia by in situ hybridization analysis. A corresponding 1.15 kb RNA mapping to the same location and transcribed from the same strand was identified in lytically infected tissue culture cells by Northern hybridization analysis. Although transcription from the complementary strand was not detected in latently infected ganglia, synthesis of a 2.9 kb immediate early RNA and a 2.6 kb early/late RNA was observed from the complementary strand in lytically infected tissue culture cells. The 3' ends of the 2.6 kb and 2.9 kb RNAs titally overlap the 1.15 kb transcript. Sequence analysis of the LR-RNA sense strand indicates the presence of two open reading frames (ORFs) within the approximate map positions of the LR-RNA. The complementary strand contains the 3' portion of an ORF that continues into the region for 1425 bases. Additional analysis indicates the presence of transcriptional regulatory sequences preceeding the LR-ORFs while codon preference analysis suggests that the LR-ORFs and the complementary ORF encode polypeptides. Together these data suggest that the LR-RNA seen in latently infected neurous overlaps the 3' end of an IE gene present on the complementary strand.

Publications: 88/01 to 88/12

MAINPRIZE, T., KUTISH, G. and ROCK, D.L. (1988). Characterization of Transcription in the Latency-Related Region of Bovine Herpesvirus Type 1. (Abstract, 13th International Herpesvirus Workshop).
DELHON, G. and ROCK, D.L. (1988). Persistent Infection with Bovine Herpesvirus Type 1 in Cultured Rabbit Sensory Neurons. (Abstract, 2nd Annual Bovine Herpesvirus Workshop).
LOKENSGARD, J.R., LEWIS, T.L., KUTISH, G. and ROCK, D.L. Molecular Characterization of Dexamethasone Induced Reactivation of Latent Bovine Herpesvirus Type 1 (BHV-1) Infection. (Submitted for publication).
MAINPRIZE, T., KUTISH, G. and ROCK, D.L. Characterization of the Latency-Related Region of the Bovine Herpesvirus Type-1 Genome. (Submitted for publication).

30.032 CRIS0094364

BIOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF BOVID HERPESVIRUS TYPE 4 (BHV-4) ISOLATES FROM CATTLE

HENRY B; School of Veterinary Medicine; University of Nevada, Reno, **NEVADA** 89557.
Proj. No.: NEV00804 Project Type: HATCH
Agency ID: CSRS Period: 06 AUG 84 to 30 JUN 87

Objectives: Determine the growth characteristics of a number of BHV-4 isolates. Analyze the genome of each. Identify the primary target cells of BHV-4. Determine the transforming potential of BHV-4. Determine the latency of the virus. Determine the presence of absence of BHV-4 defective interfering particles. Determine the degree of genetic relatedness of BHV-4 to other herpesviruses.

Approach: BHV-4 virus isolates will be grown in tissue culture, purified and analyzed by restriction enzymes. Cells of lymphoid origin will be infected and examined for virus replication in particular cell types. Transformation of lymphoid tissue will be

attempted using cells from whole blood culture in RPMI media. Virus persistence will be determined by cocultivation with known virus permissive cells.

Progress: 84/08 to 87/06. Our earlier work has focused on the genomic structure and genetic relatedness of Bovine Herpesvirus Type 4 (BHV-4). In addition to published work we are in the process of cloning and mapping the DNA of BHV-4. However, recently work in our laboratory has centered on determination of the size and number of the polypeptides present in the bovid herpesvirus type 4 (BHV-4) virion. Polyacrylamide gel electrophoresis (PAGE) assays revealed at least 33 proteins, ranging in molecular weight (MW) from 195 to 11.7 kilodaltons (Kd), were present in the purified virion of the BHV-4 prototype strain DN-599. Several of the products were present in relatively large quantity and probably represent virus structural components. One of these proteins, exhibiting an apparent MW of 155 Kd, was presumed to be the main BHV-4 structural polypeptide. Similar studies of two abortion associated virus field isolates revealed protein patterns essentially identical to DN-599. Infection of Madin-Darby bovine kidney (MDBK) cells with BHV-4 was found to significantly alter the cellular protein pattern when compared to that of control uninfected cells. PAGE analysis revealed that 9 proteins were newly synthesized or increased in relative amount while 5 proteins decreased in abundance. Additional studies were performed on extracts of cells at 1 to 8 days post-infection.

Publications: 84/08 to 87/06

EVERMANN, J.F. and HENRY, B.E. (1987). Herpetic infections of cattle: Comparative clinical and diagnostic features of bovine cytomegalovirus and infectious bovine rhinotracheitis. Comp. Cont. Educ. Pract. Vet. (In press).

HENRY, B.E., OTA, R. and EVERMANN, J.F. (1986). The genetic relatedness of disease associated field isolates of bovine herpesvirus type 4 (BHV-4). Amer. J. Vet. Res. 47:2242-2246.

EVERMANN, J.F., KENNEDY, T., CHEEVERS, P., HENRY, B.E. and BARRETT, D. (1985). Diagnostic applications of molecular epidemiology to bovine herpetic infections. 28th Annual Conference of Animal Laboratory Diagnosticians, Milwaukee, WI.

EVERMANN, J.F., HENRY, B.E., KENNEDY, T. and CHEEVERS, P. (1986). Differentiation of bovid herpesviruses of type 1 and 4 by restriction enzyme analysis. IV: Int. Sym. Vet. Lab. Diag., Amsterdam, The FNetherlands.

HENRY, B.E., OTA, R. and EVERMANN, J.F. (1986). Initial characterization of bovid herpesvirus type 4 (BHV-4) proteins. VII: West. Con. Food Animal Vet. Med. Tucson, AR.

30.033 CRIS0045857

FOOT-AND-MOUTH GENE AND ANTIGENIC STRUCTURE: EXPRESSION OF FMDV IMMUNOGENS

MOORE D M; GRUBMAN M J; KENDALL J;
Agricultural Research Service; Plum Island Animal Dis Center, Orient Point, **NEW YORK** 11944.

Proj. No.: 1940-20460-045-00D
Project Type: INHOUSE
Agency ID: ARS Period: 21 NOV 79 to 30 SEP 86

Objectives: To determine the structure (sequence) of immunogens of FMDV and determine the basis for the immune response to FMDV. To compare the variability of FMDV strains through analysis of the genome products and functions. To study and develop protein vaccines produced by chemical synthesis, through gene expression in procaryotes, eucaryotes, and in infectious virusvectors.

Approach: Identify the polypeptide sequences of virus structural and other viral encoded proteins through nucleotide sequencing of cloned viral genetic material or directly sequence selected polypeptides. Compare sequences of variants to study variation in antigenicity of FMDV. Study the basis of immunization through the preparation of experimental vaccines using poly- peptides generated by chemical synthesis, or biosynthesis in cells engineered to produce viral proteins. Immunize laboratory animals and livestock to determine immunization and protection against FMD with variouspolypeptide vaccines. Identify the location and structure of antigenic sites through competitive inhibition using selected synthetic peptides. Explore the feasibility of constructing native viral antigenic structures as vaccines through expression of viral genes in transformed cells or by viral vectored FMD genes. Study the processing of viral antigens through selective cloning techniques.--Plum Island, NY, Molec. Biol. Lab. 101-C, BL-3, 1/29/80, DM Moore/DO Morgan/MJ Grubman/JL Card/M Zellner/KH Axelson.

Progress: 86/01 to 86/12. Gene segments coding for FMDV viral protein VP-1 cloned into bacterial plasmid expression vectors synthesize high levels of the polypeptide in E. coli. Type A12 VP-1 has been extensively tested in cattle to evaluate the effectiveness of the protein vaccine. High levels of immunity were obtained in the majority of animals and reduced severity of symptoms was observed for animals which became infected on challenge of immunity. Virus type O1 VP-1 has previously generated poor immunity in livestock. Recent tests with a combined polypeptide of two areas of the VP-1 protein generated moderate protective levels in cattle vaccinated twice. In connection with antigenic analysis of VP-1 of type A and O FMDV, variants resistant to specific neutralizing monoclonal antibodies were generated. The variants are analyzed for nucleotide sequence changes in the RNA genome to pinpoint the important sites relating to immunization. The gene segment for VP-1 has been isolated and engineered into vaccinia virus as an experimental, live recombinant viral vaccine. Cell cultures infected with the recombinant virus synthesize FMDV VP-1, but

vaccination of guinea pigs, cattle and mice failed to mount an antiviral response. It is likely that the cytoplasmically located VP-1 antigen was not released or that the immunogenicity of the VP-1 as presented to the host was poor. Experiments are being extended to incorporate additional structural components to facilitate morphogenesis and increase potency.

Publications: 86/01 to 86/12
NO PUBLICATIONS REPORTED THIS PERIOD.

30.034 CRIS0140880
PRIMARY STRUCTURE OF THE FMDV GENOME AND GENERATION OF INFECTIOUS DNA CLONES

MOORE D M; WIMMER E; VAKHARIA V; Microbiology; State University of New York, Stony Brook, **NEW YORK** 11794.
Proj. No.: 1940-34000-012-04S
Project Type: COOPERATIVE AGREE.
Agency ID: ARS Period: 01 OCT 85 to 30 SEP 87

Objectives: To develop rapid methods to sequence and study the primary structure of theFMDV genome to determine function of the genome and antigenic characteristics of capsid protein antigens. To examine the transcription and translation in vitro and the expression of cDNA segments of FMDV protein coding regions in tissue cultures. To study the processing of FMDV polyproteins and evaluate the possibility of generating native structures of the capsid proteins of the virus.

Approach: Sequence data will be obtained from the genomic RNA or cloned cDNA segmentsof the FMDV genome. Specific areas of the genome will be selected for study, with the non-coding areas of the genome, the capsid protein coding regions, and coding regions for other non-structural proteins. Specialized vectors containing protein coding cDNA segments of the FMDV genome will be transcribed in vitro and subsequently translated in vitro. Vectors will be transfected into mammalian tissue culture cells to study the expression/ assembly of the capsid region. The function of non-structural proteins and the antigenicity of capsid structural proteins will be studied in this manner. The regeneration of progeny virus from cloned cDNA will be considered by transfection of either plasmids containing full-length genomeinserts or transfection of RNA transcripts of cDNA clones.

Progress: 88/01 to 88/12. Previously, in vitro transcription/translation systems were used to determine the requirements for expression and proteolytic processing of the capsid polyprotein precursor molecule into the individual capsid proteins. To examine the processing of FMDV proteins expressed in vivo, two transient expression systems were developed. Different segments of the coding sequence of the FMDV genome were cloned into plasmids containing either the bacteriophage T7 promoter or a vaccinia late promoter. These were transfected into tissue culture cells infected with a recombinant vaccinia virus expressing the T7 RNA polymerase or with wild type vaccinia virus WR, respectively. Cells were harvested and extracts of the cells were analyzed by western blot analysis with a VP1 antiserum/125I-protein A detection system. The results showed that the P1-2A region was efficiently cleaved from precursor polyprotein and that the P1 region was further processed to capsid polypeptides if the clones contained the coding sequence for the viral protease, 3C. The results indicate that FMDV proteins expressed under the control of the T7 promoter or a vaccinia promoter can be effectively processed into capsid proteins in vivo and that stable vaccinia virus recombinants should be able to be engineered to express the same proteins. Work is underway to engineer and isolate such recombinant vaccinia viruses.

Publications: 88/01 to 88/12
VAKHARIA, V.N., DEVANEY, M.A., GRUBMAN, M.J., and MOORE, D.M. 1988. Cloning and expression of foot-and-mouth disease virus genes. XI Pan American Congress of Veterinary Sciences. Lima, Peru. (Abstract).

30.035 CRIS0096210
MOLECULAR ANALYSIS OF LATENT INFECTION WITH BOVINE HERPESVIRUS TYPE 2 (BHV-1)

ROCK D L; Veterinary Science; North Dakota State University, Fargo, **NORTH DAKOTA** 58105.
Proj. No.: ND05143 Project Type: SPECIAL GRANT
Agency ID: CSRS Period: 01 AUG 85 to 30 SEP 87

Objectives: The proposed specific objectives and approaches include: 1) identifying, with in situ hybridization, those regions of the BHV-1 genome that are transcriptionally active in neurons during the latent phase of infection and during the early stages of dexamethasone induced viral reactivation and 2) identifying and mapping latency-related viral genes located within identified transcriptionally active regions using RNA hybrid selection/in vitro translation, RNA filter hybridization, and cross hybridization studies with herpes simplex virus type 1 (HSV-1).

Approach: See Objectives.

Progress: 85/08 to 87/09. BHV-1, like all other members of the herpesvirus group, establishes latent infections. In all likelihood it is this mechanism alone that is responsible for perpetration and transmission of infection in cattle. BHV-1 latency related RNA from latently infected cells was identified and fine mapped using in situ hybridization and standard cloning techniques. RNA probes will be used to determine the direction DNA is being transcribed which will be useful to determine if it is the sense or antisense strand of a potential gene as has been found in the HSV-1 system with the ICP-0 gene. Gene transcription is restricted to this latency related area of the BHV-1 genome in latent cells. Reactivation of BHV-1 in latently infected rabbits was achieved by a single intravenous dose of dexamethasone or topical application of prednisolone. A shift from the viral transcriptional pattern characteristic of the

latent state to one typical of the acute infection was detected between 15 and 18 hours post treatment with viral DNA synthesis first detectable at 18 to 21 hours. Quantitative experiments were performed using probes indicative of the latent state and also the reactivated state on serial sections of trigeminal ganglia from reactivated latent rabbits. There was a statistically significant decrease in the number of cells that hybridize with the latent probe at 48 hours post treatment. This depression may play an important regulatory role in the establishment, maintenance, and reactivation of the latent state.

Publications: 85/08 to 87/09

ROCK, D.L., BEAM, S.L., and MAYFIELD, J.E. 1987. Mapping Herpesvirus Type I Latency. Related RNA in Trigeminal Ganglia of Latently Infected Rabbits. J. of Virology 61:3827-3831.

ROCK, D.L., NESBURN, A., CHIASI, H., ONG, J., LEWIS, T.L., LOCKENSGARD, J.R., and WECHSLER, S. 1987. Detection of Latency-Related Viral RNAs in TG Rabbits Latently Infected with Herpes Simplex Virus Type 1. J. Virol. 61:3820-3826.

ROCK, D.L., HAGEMOSE, W.A., OSORIO, F.A., and REED, D.E. 1986. Detection of Bovine Herpesvirus Type I RNA in TG of Latently Infected Rabbit by in situe Hybridization. J. Gen. Virol. 67:2515-2520.

30.036 CRIS0130202

NUTRIENT UTILIZATION IN BRUCELLA ABORTUS STUDIED BY CLONING THE GENES IN E. COLI

ESSENBERG R C; Biochemistry; Oklahoma State University, Stillwater, **OKLAHOMA** 74078.
Proj. No.: OKL01993 Project Type: CRGO
Agency ID: CRGO Period: 01 SEP 86 to 28 FEB 90

Objectives: PROJ 8601136. To study carbohydrate, specifically glucose, transport and utilization in Brucella abortus and the adaptations made in these processes to allow growth and division inside macrophages.

Approach: We will study nutrient uptake and utilization, specifically glucose, by cloning the genes from Brucella into E. coli. They will be detected by complementation of E. coli mutants defective in glucose transport or other genes of glucose metabolism. The resulting plasmids will be cut down to the minimum size required for expression of the desired character, and the expression maximized, if necessary, by coupling the gene to good promoters in E. coli. The properties of the transport system will be studied in E. coli to determine such things as the mechanism of energy coupling and the identity of the protein involved, using methods developed for studies in E. coli. The control of the system will be studied by testing the effect of things like bovine placental extracts, pH and Ca concentration on the activity of the system and its expression.

Progress: 87/10 to 88/09. The clones carrying the pro A and leu B complementing ability have been mapped in detail by restriction enzyme digestion, and the location of the genes determined by recloning fragments. These fragments have been used to hybridize with Brucella genomic DNA digests to show that the clones and genome have the same fragments. The clones complementing the glucose utilization defect have been studied in a similar manner. The most stable and active one has been assayed for glucokinase and has definite activity. The apparently unstable clones were found to result at least in part from having more than one plasmid. It has been possible to separte these plasmids. The strains show very weak complementation, but appear to be stable. Some of them appear to have glucokinase activity as well. The restriction maps of these various clones show no obvious similarity, which may suggest that Brucella has multiple genes for this enzyme. Several libraries have been made, using different enzymes, and screened for complementation against several other amino acid synthesis mutants and phosphogulose isomerase and glucose-6 P dehydrogenase mutants. So far, no more complementing plasmids have been found.

Publications: 87/10 to 88/09

NO PUBLICATIONS REPORTED THIS PERIOD.

30.037 CRIS0133670

DEVELOPMENT OF BHV1 AS A BOVINE RESPIRATORY VACCINE VECTOR

EBERLE R; D'OFFAY J M; FULTON R W; Veterinary Medicine; Oklahoma State University, Stillwater, **OKLAHOMA** 74078.
Proj. No.: OKL02034 Project Type: ANIMAL HEALTH
Agency ID: CSRS Period: 01 JAN 88 to 30 SEP 93

Objectives: Construct a BHV1 strain as a vaccine vector. Delete the DNA sequences coding for a non-essential glycoprotein of BHV1 and determine the properties of the gene-deletion virus in vitro and in vivo.

Approach: Locate the BHV1 glycoprotein gene using a plasmid containing a cDNA from the related PRV gIII glycoprotein. Clone and sequence the gene and adjacent non-coding sequences. Delete the gIII coding sequences by hybridization of synthetic oligodeoxynucleotide to single-stranded, cloned DNA in M13 vector. Incorporate the gIII gene deletion into BHV1. The replicative ability of the gene virus will be quantitatively assessed, and its failure to express any polypeptides antigenically related to gIII confirmed. Test the immune response to the gIII virus in rabbits.

30.038 CRIS0091297
INFECTIOUS BOVINE RHINOTRACHEITIS VIRUS: NEW APPROACHESOF LATENT INFECTIONS

LAWRENCE W; Pathobiology; University of Pennsylvania, Philadelphia, **PENNSYLVANIA** 19104.
Proj. No.: PENV-5-27732
Project Type: SPECIAL GRANT
Agency ID: CSRS Period: 01 SEP 83 to 31 AUG 87

Objectives: To use DNA hybridization procedures for detection of latent IBR viral DNA in bovine tissues; to investigate the frequency of naturally occurring latent IBR infections in the cattle population, and to correlate serologica status of cattle with the occurrence of latent infections; to determine which body tissues may harbor latent virus; to investigate the physical state of the latent IBR viral genome.

Approach: Utilizing the Southern blot technique, DNA extracted from bovine tissues will be examined for the presence of latent virus by hybridization with 32p-labeled nick translated viral DNA probes.

Progress: 85/09 to 86/08. A panel of monoclonal antibodies to IBR virus has been produced and characterized. One of these specifically indentifies a 130K glycoprotein which bears neutralizing epitopes. In addition, we have isolated a collection of monoclonal antibody resistant mutants (mar mutants) of IBR virus which can be readily distinguished from wild type virus in an immunoreactive plaque assay. By using marker rescue of IBR mar mutants with cloned wild type DNA fragments, the map location of the gene for the 130K viral glycoprotein has been mapped to within a 3.8 kb DNA fragment at map units 0.405 - 0.432 of the viral genome. Furthermore, an herpes simplex type 1 glycoprotein B specific DNA probe was found to uniquely hybridize to this same 3.8 kb fragment. These results indicate that there is significant DNA homology between HSV gB-1 and the IBR 130K glycoprotein. A bromodeoxyuridine (BU) resistant thymidine kinase (TK) negative bovine kidney cell line has been developed. By cotransfection marker rescue experiments utilizing infectious TK negative IBR DNA and cloned Hind III wild type IBR DNA fragments, and using a radioactive plaque assay to detect rescued wild type progency virus, the IBR TK gene has been mapped to a position within the Hind III A fragment.

Publications: 85/09 to 86/08
LAWRENCE, W.C., D'URSO, R.C., KUNDEL, C.A., WHITBECK, J.C., and BELLO, L.J. Map location of the gene for a 130,000 Dalton glycoprotein of bovine herpesvirus 1. Eleventh International Herpesvirus Workshop, Leeds, England, 1986., p. 302.
LAWRENCE, W.C., D'URSO, R.C., KUNDEL, C.A., WHITBECK, J.C., and BELLO, L.J. 1986. Map location of the gene for 130,000 Dalton glycoprotein of bovine herpesvirus 1. J. Virol. 60: 405-414.

30.039 CRIS0130495
DEVELOPMENT OF BOVINE HERPESVIRUS I AS EXPRESSION VECTOR FOR GENES OF OTHER BOVINE PATHOGENS

LAWRENCE W; BELLO L; School of Veterinary Medicine; University of Pennsylvania, Philadelphia, **PENNSYLVANIA** 19104.
Proj. No.: PENR-5-20459 Project Type: CRGO
Agency ID: CRGO Period: 01 SEP 86 to 31 AUG 89

Objectives: Project 8601420. To develop sufficient new information concerning the molecular biology of BHV1 to allow the use of this virus as a vector which expresses genes of other bovine pathogenic organisms and which may be of value as an effective live attenuated polyvalent vaccine.

Approach: Insertion vectors will be constructed such that inserted foreign genes will be expressed utilizing either the Tk or g 130 promotor and regulatory sequences. These insertion vectors will then be used to insert foreign genes into BHV1 and the resulting recombinants will be tested for expression of foreign genes and for their ability to stimulate an immune response to proteins expressed by the inserted foreign genes.

Progress: 88/01 to 88/12. The complete nucleotide sequence of the BHV1 gI gene and the predicted 932 amino acid sequence of the gI primary translation product were determined. Comparison with the published nucleotide sequence of the HSV-1 ?KOS? gB gene revealed a similarity of 56.3% at the nucleotide level and 45.9% at the amino acid level. Upstream of the proposed gI coding region are potential mRNA transcriptional promoter elements including a TATA box and multiple Sp1 binding sites (GC boxes). Downstream of the gI coding region are two sequence elements associated with mRNA cleavage/polyadenylation (AATAAA and a GT-rich region roughly 30 nucleotides further downstream). Like HSV-1 gB, the predicted gI amino acid sequence exhibits two broad hydrophobic regions likely to represent a transient amino terminal signal sequence and a transmembrane anchor domain (near the carboxyl-terminus). Additional features shared with gB include 6 potential N-linked glycosylation sites and 10 highly conserved cysteine residues in the gI extracellular domain. Two regions of non-similarity between gI and gB are a centrally located 22 amino acid region of gI for which there is essentially no gB counterpart and the transient amino terminal leaders which differ in both size and sequence. The hydrophobic signal sequence of the gI leader, unlike that of gB, is preceded by an unusually large region of predominantly hydrophilic amino acids.

Publications: 88/01 to 88/12
BELLO, L.J., J.C. WHITBECK and W.C. LAWRENCE. (1987). Map location of the thymidine kinase gene of the bovine herpesvirus 1. J. Virol. 61: 4023-4025.
WHITBECK, J.C., L.J. BELLO and W.C. LAWRENCE. (1988). Comparison of the bovine hervesvirus 1 gI gene and the herpes simplex virus type 1 gB gene. J. Virol. 62:3319-3327.

30.040 CRIS0097184
CLONING AND HYBRIDIZATION PROBES IN PATHOGENESIS STUDIES

POTGIETER L N D; Pathobiology; University of Tennessee, Knoxville, **TENNESSEE** 37996.
Proj. No.: TEN000782Project Type: SPECIAL GRANT
Agency ID: CSRS Period: 15 SEP 85 to 30 SEP 88

Objectives: To purify BVD virus; extract RNA and nick translate the RNA. To clone a library of cDNA into plasmid vectors. To produce large quantities of BVD virus DNA from plasmid DNA. To produce biotinylated probes from BVD virus DNA. To test biotinylated probes in cell cultures and infected calves.

Approach: BVDV will be grown on bovine deximal cells and purified by density gradient centrifugation. RNA will be extracted with phenol and nick translation done with reverse transcriptase. Cloning will be done by restriction endonuclease digestion of cDNA followed by insertion into pUC-9 plasmid vector. Plasmid DNA will be harvested from broth cultures of E. coli and BVDV DNA extracted with restriction endonucleases and electrophoresis. Probes will be produced by nick translation in the presence of biotinylated nucleotides. Probes will be tested in appropriate materials by immunofluorescence and dot blot ELISA.

Progress: 88/01 to 88/12. RNA from a pneumopathic strain of BVDV was purified from infected cell culture fluids and tailed with adenine residues at the 3' end with poly-A polymerase. Double-stranded cDNA was synthesized, using the poly-A-tailed RNA as a template and oligo dT as a primer, and then cloned into a pUC9 plasmid. Virus-specific cDNA, varying in length from 0.5 to 2.5 kb, was obtained. One BVDV-specific sequence of cloned cDNA, 1.1 kb in length and with an internal Pst I restriction endonuclease cleavage site, was selected for use as a probe. The cloned cDNA insert was removed from the plasmid either with or without flanking plasmid sequences and labelled with (superscript 32)P-nucleotides by nick translation for use as hybridization probes for BVDV. The performance of probes of various lengths was evaluated. In addition two methods of specimen preparation were compared to establish optimum parameters for hybridization. The hybridization assay was 10 to 100 times more sensitive than infectivity assays for BVDV in infected cell cultures. Freezing of specimens reduced by 10-fold the sensitivity of the hybridization for BVDV target sequences. The probes prepared from the cloned cDNA hybridization with all cytopathic and noncytopathic BVDV strains tested but not with uninfected cell cultures, cellular ribosomal RNA, bovine coronavirus, bluetongue virus or bovine adenovirus 3. Probes prepared from native plasmid DNA did not hybridize with BVDV or uninfected cell cultures. Probes 1.1, 0.6, and 0.

Publications: 88/01 to 88/12

BROCK, K.V., BRIAN, D.A., ROUSE, B.T., and POTGIETER, L.N.D. Molecular cloning of cDNA from a pneumopathic strain of bovine viral diarrhea virus and its diagnostic application. Can J Vet Res 52:451-457, (1988).

POTGIETER, L.N.D. and BROCK, K.V. Molecular cloning of bovine viral diarrhea virus cDNA and prospects for recombinant vaccines. Proc U.S. Anim Hlth Assoc 91:203-215, (1987).

POTGIETER, L.N.D. and BROCK, K.V. Detection of bovine viraldiarrhea virus by spot hybridization with probes prepared from cloned cDNA sequences. J Vet Diagn Invest 1:172-188, (1988).

POTGIETER, L.N.D. Strategies for the control of bovine viral diarrhea virus infection in beef and dairy cattle. Proc Symp on Bov Viral Diarrhea, Univ of Montreal, St Hyacinthe, pp 71-92, Oct (1987).

30.041 CRIS0130960
BOVINE RESPIRATORY DISEASES: RISK FACTORS, PATHOGENS, DIAGNOSIS AND MANAGEMENT

POTGIETER L N D; Pathobiology; University of Tennessee, Knoxville, **TENNESSEE** 37996.
Proj. No.: TEN00821 Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 86 to 30 SEP 91

Objectives: Prepare clones of BVD virus CDNA containing full and partial genomic segments. Use hybridization to detect viral genetic material in cell cultures and in clinical materials within 2 hours with great sensitivity and specificity. Prepare Pasteurella haemolytica vaccine which elicits high levels of leukotoxin-neutralizing antibodies. Investigate the various P. haemolytica pathogenic vactors and interactions with the immune system. Accurately assay the prevalence of respiratory disease in Tennessee beef herds. Determine whether stimulation of gut associated lymphoid tissue with P. haemolytica is a useful avenue of vaccination.

Approach: cDNA will be prepared from purified BVD virus RNA, polyadenylated and cloned in the puc-9 vector of E. coli. Size of the clones will be measured by PAGE analysis. Cloned DNA will be prepared and biotinylated for use as a probe. In situ and dot blot hybridization with Fluorescence and ELISA detection of the label will be done with infected cell cultures and clinical materials. Bacterium-free P. haemolytica preparations will be tested in rabbits initially for induction of leukotoxin-neutralizing antibodies. In vitro systems (lung explant cultures, tracheal outgrowth cultures) will be used to test in vitro the evidence that neutrophil interaction is important in the pathogenesis of pasteurellosis.

Progress: 88/01 to 88/12. Work on the molecular cloning, genetic organization and application of cloned cDNA sequences of BVDV is continuing. The performance of radioisotope probes prepared from BVDV cDNA was evaluated. The hybridization assay was 10 to 100 times more sensitive than infectivity assays for BVDV in infected cell cultures. Freezing of specimens reduced by 10-fold the sensitivity of the hybridization for BVDV target sequences. The probes were specific for BVDV. Probes 1.1,

0.6, and 0.5 kb hybridized with equal sensitivity to target sequences in the dot blot system. The presence of flanking plasmid sequences did not improve the hybridization performance of the probes. Hybridization results with specimens prepared by RNAsin and heat treatment were similar to those obtained after nonidet P-40 and formaldehyde treatment. Hybridization studies of animals undergoing primary postnatal infections indicates that the virus persists much longer in lymphocytes than previously suspected. These assays are much superior to immunoassays for detecting certain noncytopathic strains of BVDV from clinical materials and biologics. Recent work has demonstrated that Pasteurella haemolytica crude leukotoxin has a similar detrimental effect on endothelial cells with or without the presence of neutrophils. These effects on endothelial cells have a different kinetic pattern than the acute cytotoxicity (less than one hour) observed in leukocytes.

Publications: 88/01 to 88/12

POTGIETER, L. N. D., BROCK, K. V., and BRIAN. D. A. Use of hybridization probes prepared from cloned cDNA sequences for the detection of bovine viral diarrhea viruses. Abstract. Proc Am Assoc Vet Lab Diagnost 30:36, (1987).

BROCK, K. V., POTGIETER, L. N. D, and BRIAN, D. A. Hybridization probes from cloned bovine viral diarrhea virus cDNA sequences. Abstract. Proc Conf Res Workers Anim Dis 68:56, (1987).

POTGIETER, L. N. D., HELMAN, R. G., GREENE, W., BREIDER, M. A., THURBER, E. T., and PEETZ, R. H. Experimental bovine respiratory tract disease with Haemophilus somnus. Vet Pathol 25:124-130, (1988).

BROCK, K. V. and POTGIETER, L. N. D. Rapid fluorescence detection of in situ hybridization with biotinylated bovine herpesvirus 1 DNA probes. J Vet Diagn Invest 1:34-38, (1988).

BREIDER, M. A., WALKER, R. D., HOPKINS, F. M., SCHULTZ, T. W., and BOWERSOCK, T. L. Pulmonary lesions induced by Pasteurella Haemolytica in neutrophil sufficient and neutrophil deficient calves. Can J Vet Res 52:295-309, (1988).

BREIDER, M. A. and RUX, T. L. Effect of Pasteurella Haemolytica Leukotoxin and bovine neutrophils on bovine endothelial cells. Abstract. Proc Intern Symp Virulence Mechanisms Vet Bact Pathogens, Ames, IA, June (1987).

PRINGLE, J. K., VIEL, L., SHEWEN, P. E., WILLOUGHBY, R. A., MARTIN, S. W., and VALLI, V. E. O. Bronchoalveolar lavage of cranial and caudal lung regions in se.

30.042 CRIS0136473

GENETIC ORGANIZATION OF A PNEUMOPATHIC STRAIN OF BOVINE VIRAL DIARRHEA VIRUS

POTGIETER L N D; Pathobiology; University of Tennessee, Knoxville, **TENNESSEE** 37996.
Proj. No.: TEN00886 Project Type: SPECIAL GRANT
Agency ID: CSRS Period: 01 SEP 88 to 31 AUG 91

Objectives: Objectives are to 1. Construct a structural map of the BVDV virus genome. To sequence the genome to identify individual genes; to subclone individual putative BVDV genes into vaccinia virus expression vectors. To evaluate the expression of individual BVDV genes in recombinant vaccinia virus-infected cell cultures.

Approach: The structural map of the BVDV genome will be done by cross hybridization among cloned cDNA sequences which have already been obtained in our laboratory. The genome will be seququenced by the dideoxy nucleotide chain termination method adapted for sequencing DNA cloned into pUC vectors according to the manufacturers instructions. Complete open reading frames will be subcloned into vaccinia recombinants by methods that are now standard. The expression of gene products in cell cultures infected with the recombinant vaccinia constructs will be evaluated by immunofluorescence and immunoprecipitation.

Progress: 88/09 to 88/12. Work has just begun on this new project we have identified five cDNA clones representing five separate but overlapping areas of the BVDV genome and have started sequencing them using the dideoxy chain termination procedure using the sequenase system. Probes for crosshybridization were prepared by nick translation for (superscript 32) P incorporation. The procedure has allowed us to classify the cloned cDNA plasmid inserts into 5 distinct overlapping regions.

Publications: 88/09 to 88/12

No publications reported this period.

30.043 CRIS0142366

PRODUCTION OF A RECOMBINANT SUBUNIT VACCINE FOR THE PROTECTION OF CATTLE AGAINST HYPODERMA SP.

PRUETT J H; KIRSCHBAUM J B; Agricultural Research Service, Kerrville, **TEXAS** 78028.
Proj. No.: 6205-35000-003-01T
Project Type: INHOUSE
Agency ID: ARS Period: 30 JUL 88 to 30 JUL 89

Objectives: To produce recombinant antigen(s) derived from proteins obtained from Hypoderma lineatum, the common cattle grub, for the purpose of producing a genetically engineered subunit vaccine.

Approach: The established immunological, molecular (recombinant DNA), and biochemicaltechniques will be used to isolate pure hypodermin A and mRNA which encodesfor hypodermin A. Hypodermin A will be partially sequenced and mixed oligonucleotide probes will be constructed for cloning and sequencing the corresponding gene. cDNA for hypodermin A will be prepared and cloned. Expression will be optimized for vaccine production. Recombinant antigen(s)will be evaluated for comparative immunogenicity with natural hypodermin A along with dose-dependent efficacy studies. INCOMING FUNDS TO ARS FROM CODON.

Progress: 88/01 to 88/12. Work by the USDA-ARS and CODON through a cooperative agreement to produce a recombinant vaccine for control of the cattle grub, Hypoderma lineatum, has been underway for 1 year. CODON provided USDA-ARS with the first recombinant construct of hypodermin A for testing of immunological properties and efficacy in February 1988. Two additional recombinant proteins were produced by CODON and subjected to testing by USDA-ARS in July 1988. The humoral and cellular immunity of these three proteins in combination with various veterinary acceptable adjuvants are being evaluated. Efficacy data from these trials will be available for the first time in March 1989.

Publications: 88/01 to 88/12
NO PUBLICATIONS REPORTED THIS PERIOD.

30.044 CRIS0132970
MOLECULAR AND SOMATIC CELL GENETICS: MAPPING THE CATTLE GENOME

WOMACK J E; Veterinary Pathology; Pb Box 3578, College Station, **TEXAS** 77843.
Proj. No.: TEX06912 Project Type: CRGO
Agency ID: CRGO Period: 01 SEP 87 to 31 AUG 90

Objectives: To map cattle genes of key physiological significance, including the genes coding immunoglobulins, prolactin, parathyroid hormone, fibronectin, homeotic boxes & interferons. To determine the extent and nature of variation in these genes in breeding populations of cattle. To determine the molecular organization and natural genetic variability of the interferon, immunoglobulin, and MHC multigene families. Proj. 8701679.

Approach: The combined technologies of somatic cell and molecular genetics will be utilized to produce both a genetic and a physical map of the cattle genome. A large pedigreed herd will be tested for restriction fragment length polymorphisms (RFLPs) to evaluate the genetic diversity in key genes & gene families and to establish a genetic (recombination) map.

Progress: 88/01 to 88/12. The objectives of this project are to utilize somatic cell genetic methods to generate a gene map of the cow and to expand this map by isozyme, DNA fragment, and karyotypic analysis. Restriction fragment length polymorphisms are being discovered that will permit the combination of recombinant mapping in meiosis with synteny mapping in somatic cells. The ultimate objective is to utilize the map to define markers of disease resistance and productivity. A total of 12 DNA polymorphisms have been discovered, including genes for osteonectin, gamma crystallin, and 21-steroid hycroxylase (not reported previously). Each of these has been added to the synteny map by analysis of hybrid somatic cells. The total number of genes mapped in cattle is now over 100 with 8 assignments of syntenic groups to specific chromosomes.

Publications: 88/01 to 88/12
WOMACK, J.E. Genetic engineering in agriculture: animal genetics and development. Trends in genetics 3:65-68, 1987.
WOMACK, J.E. A gene map of the cow. Genetics Maps 4:499-501, 1987.
ADKISON, L.R., LEUNG, D.W. and WOMACK, J.E. Somatic cell mapping and restriction fragment analysis of bovine alpha and beta interferon gene families.
Cytogenet. and Cell Genet. 47:62-65, 1988.
WOMACK, J.E. Comparative gene mapping: A valuable new tool for mammalian developmental studies. Developmental Genet. 8:281-293, 1987.
WOMACK, J.E. Molecular cytogenetics of cattle: a genomic approach to disease resistance and productivity. J. Dairy Sci. 71:1116-1123, 1988.
MCAVIN, J.C., PATTERSON, D. and WOMACK, J.E. Mapping the bovine PRGS and PAIS genes in hybrid somatic cells: syntenic conservation with human chromosome 21.
Biochem. Genet. 26:9-18, 1988.
SKOW, I.C., WOMACK, J.E., PETRASH, J.M. and MILLER, W.L. Synteny mapping of bovine genes for 21 steroid hydroxylase, alpha A-crystallin and class I bovine leucocyte antigen (BoLA) in cattle. DNA 7:143-149, 1988.

30.045 CRIS0097956
MOLECULAR GENETICS OF CYTOCHROME P-450 GENE FAMILIES IN CATTLE

SKOW L C; Veterinary Anatomy; Texas A&M University, College Station, **TEXAS** 77843.
Proj. No.: TEX06831 Project Type: ANIMAL HEALTH
Agency ID: CSRS Period: 14 FEB 86 to 30 SEP 88

Objectives: To establish the chromosomal locations of four families of cytochromes P-450 in cattle and identify restriction endonuclease fragment polymorphisms in cytochrome P-450 genes among eight breeds of cattle for use as gene markers for P-450 genotypes in subsequent experiments to correlate P-450 genotypes with levels of xenobiotic metabolism.

Approach: Bovine cytochrome P-450 genes will be assigned to chromosomes by DNA hybridization analysis of bovine x hamster somatic cell hybrid clones. Genetic variation in bovine cytochrome P-450 genes will be detected as restriction fragment length polymorphisms in DNA prepared from lymphocytes or semen collected from an experimental cattle herd maintained by the Texas Agricultural Experiment Station (H6749).

Progress: 86/01 to 88/09. The objective of this project is: to define genetic markers for various genes of the cytochrome P-450 monoxygenase system; to better understand the organization and distribution of these genes in the bovine genome; and, to develop genetic systems to search for cytochrome P450-mediated toxicity. Clones of seven genes have been acquired as cDNAs, including: 11 beta, 15 yield, 17 yield, and 21 steroid hydroxylases, adrenodoxin, side chain cleavage enzyme, and

the phenobarbital and methylcholanthrene-inducible forms of P-450. We have identified the chromosomal location of 21OH and defined RFLPs for 21OH, 17 yield and SSC in cattle. These studies will continue as planned until each of the P-450 genes identified abouve has been genetically characterized in cattle.

Publications: 86/01 to 88/09
SKOW, L.C., WOMACK, J.E., PETRESH, J.M. and MILLER, W.C. 1988. The genes for 21 steriod hydroxylase and (d)A-crystalline are not syntenic in cattle. DNA. 7:143-149.

30.046 CRIS0135643
MOLECULAR GENETICS AND MAPPING OF DISEASE RESISTANCE GENES IN CATTLE

SKOW L C; Veterinary Anatomy; Texas A&M University, College Station, **TEXAS** 77843.
Proj. No.: TEX06923 Project Type: HATCH
Agency ID: CSRS Period: 22 AUG 88 to 31 JUL 93

Objectives: 1. Establish the chromosomal locations of loci for the major histocompatibility complex, macrophage function, and various forms of cytochrome P-450 monooxygenases in cattle. 2. Identify genetic polymorphisms among cattle that may be correlated with predisposition to diseases.

Approach: Recombinant DNA techniques will be used to analyze somatic cell hybrid clones from bovinexhamster cell fusions to assign bovine genes to chromosomes. Clones of different genes from humans, mice or cattle will be 32P-labelled and hybridized to DNA from somatic cell hybrid cell lines that have randomly lost cattle chromosomes. The concordant presence or absence of gene sequences with specific chromosomes will be used to assign genes to cattle chromosomes. Intraspecies variation in disease resistance genes will be detected by analysis of DNA from pedigreed cattle in multibreed herds supported by TAES (H6749). DNA will be analyzed by restriction endonucleases and blot hybridization with radioactive gene probes to detect restriction fragment length polymorphisms (RFLP).

Progress: 88/01 to 88/12. A major research objective in animal agriculture is the development of genetic approaches to increase disease resistance in cattle. Several bovine genes and gene families have been targeted for analysis based on reported disease association of homologous genes in other species, notably mice and humans. Genes being studied include the major histocompatibility complex (BoLA), several genes of the cytochrome P-450 mono-oxygenase system, gamma crystallin and fibronectin. The latter two genes are closely linked to a gene conferring resistance to a broad range of intracellular pathogens in mice. Restriction fragment length polymorphisms have been identified for each of these genes in cattle and are being used as gene markers to search for disease resistance genes. Recently, we completed genetic analysis of a herd of cattle demonstrating brucellosis resistance. Fifteen gene markers have been developed in this herd of 148 animals, and data are presently being analyzed for correlation with brucellosis resistance.

Publications: 88/01 to 88/12
ADKISON, L., SKOW, L.C., THOMAS. T.L., PETRESH, J.M. AND WOMACK, J.E.: 1988. Somatic cell mapping and restriction fragment analysis of bovine genes for fibronectin and gamma crystallins. Somat. Cell Genetics. 47:155-159.
SKOW, L.C., DONNER, M.E., HUANG, S-M., TAYLOR, B.A., BEAMER, W.G., AND LALEY, P.A.: 1988. Gene sequences for mouse gamma crystallins are on chromosome 1. Biochem. Genet. 26:557-570.
BEAMER, W.G., TENNENT, B.J., SCHULTZ, K.L., NADEAU, J.H., SCHULTZ, N.L. and SKOW, L.C.: 1988. Gene for Ovarian Granulosa Cell Tumor Susceptibility, GCT, in SWXJ Recombinant Inbred Strains of Mice Revealed by Dehydroepiandrosterone.

30.047 CRIS0136233
ORGANIZATION AND EXPRESSION OF BOLA CLASS I GENES

SKOW L C; Veterinary Anatomy; Texas A&M University, College Station, **TEXAS** 77843.
Proj. No.: TEX06958 Project Type: CRGO
Agency ID: CRGO Period: 01 SEP 88 to 31 AUG 91

Objectives: PROJ. 8801608. Define the organization and arrangement of selected genes within the major histocompatibility complex (MHC) of cattle (BoLA). Identify genetic polymorphisms among cattle that may be correlated with predisposition to diseases.

Approach: Pulsed field gel electrophoresis will be used to analyze large (10 bp) DNA fragments in order to establish an extended restriction map of the BoLA region. Organization of BoLA genes can then be compared to the homologous regions in humans and mice to better understand the role of the MHC in disease. Intraspecies variation in disease resistance genes will be detected by analysis of DNA from pedigreed cattle in multibreed herds supported by TAES (H6749). DNA will be analyzed by restriction endonucleases and blot hybridization with radioactive gene probes to detect restriction fragment length polymorphisms (RFLP). RFLPs will be used to determine linkage relationships and to identify genotypes for additional experiments.

Progress: 88/09 to 88/12. The major histocompatibility complex (MCH) consists of a large number of closely-linked genes that encode cell-surface glycoproteins known as histocompatibility antigens. Genes of the MHC are involved in regulation of cell and humoral immune response and variant MHC genes have been associated with a variety of disease susceptibilities in humans and mice. Genetic analysis of the bovine MHC (called BoLA) is an important objective in understanding genetic predisposition to disease in cattle. We have tentatively assigned the BoLA complex to chromosome syntenic group U21 based on linkage studies using the flanking gene markers,

glyoxylase and 21 steroid hydroxylase. Eleven clones of BoLA Class I DNA sequences have been isolated and are being characterized to determine gene expression and organization of the bovine MCH complex. Restriction enzyme analysis of bovine genomic DNA has identified a large number of BoLA restriction fragment length polymorphisms which are being used to confirm the chromosome assignment and to further investigate the role of BoLA in cattle diseases.

Publications: 88/09 to 88/12

SKOW, L.C., WOMACK, J.E., PETRESH, J.M. and MILLER, W.C. 1988. The genes for 21 steroid hydroxylas and A-crystallin are not syntenic in cattle. DNA. 7:143-149.

30.048 0097881

BLUETONGUE AND EPIZOOTIC HEMORRHAGIC DISEASES IN RUMINANTS

COLLISSON E W; LIVINGSTON C W; Veterinary Microbiology & Parasitology; Texas A&M University, College Station, **TEXAS** 77843.
Proj. No.: TEX06808 Project Type: HATCH
Agency ID: CSRS Period: 01 FEB 86 to 31 JAN 91

Objectives: To develop recombinant DNA probes for bluetongue (BT) and epizootic hemorrhagic disease (EHD) viruses. To develop techniques for using these probes as a practical diagnostic tool for the clinical laboratory. To use these probes to study the pathogenesis of these viruses in the acutely infected chick embryo, sheep, and acutely and persistently infected cattle. To study the molecular epizoology of BTV and EHDV. To determine the indigenous serotypes of BTV and EHDV in Texas for future control programs. To determine gene assignments of EHDV.

Approach: Recombinant DNA techniques will be used to develop probes for detection of BTV and EHDV genomes in infected tissues. Characterization of viral genomes will be done by such techniques as polyacrylamide gel electrophoresis (PAGE) and oligonucleotide fingerprinting, whereas proteins will be analyzed by HPLC in addition to PAGE. The extent of the diversity of BTV in Texas will be determined by monitoring seroconversion of sentinel cattle and doing retrospective isolation of the viral strains. Genome reassortment procedures, as well as in vitro translation techniques will be used to map EHDV genes.

Progress: 88/01 to 88/12. In situ cytohybridization was used to determine the tissue tropism and target cells for replication of bluetongue virus (BTV) in the developing chicken embryo. Hybridization with a biotinylated probe detected viral replication in embryos inoculated with the four U.S. serotypes and a BTV field strain. At the final stages of infection, when the embryos were hemorrhagic, viral infection could consistently be detected in the brain, kidney, spinal cord, heart, lung and liver with the brain and kidney most severly affected. Other tissues, such as the retina, skin, tongue and intestinal villi also supported viral replication with greater concentration of virus localized with in epithelial cells, such as those lining the kidney tubules and tertiary bronchi of the lungs. Within 24 hours after inoculation, viral replication occurs initially in the brain and kidney. By 48 hours, viral replication can also be detected in the lungs, heart and spinal cord with the liver being severely infected by 72 hours. Low levels of hybridization could be detected in embryos infected with EHDV.

Publications: 88/01 to 88/12

WANG, L., KEMP, M., ROY, P. and COLLISSON, E. 1988. Tissue tropsim and target cells of bluetongue virus in the chicken embryo. J. Virol. 62: 887-893.

COLLISSON, E., WANG, L., ROY, P. and KEMP, M. 1988. Detection of BTV in infected chicken embryos by in situ hybridization in orbiviruses and birnaviruses in the Proc. Double Stranded RNA Virus Symposium.

30.049 CRIS0130274

MOLECULAR DIFFERENTIATION OF NONCYTOPATHIC AND CYTOPATHIC ISOLATES OF BOVINE VIRAL DIARRHEA VIRUS

COLLISSON E; KEMP M; SNEED L; Veterinary Microbiology & Parasitology; Texas A&M University, College Station, **TEXAS** 77843.
Proj. No.: TEX06864 Project Type: SPECIAL GRANT
Agency ID: CSRS Period: 01 SEP 86 to 28 FEB 90

Objectives: Cytopathic and noncytopathic isolates of bovine viral diarrhea virus (BVDV) have recently been shown to have synergistic properties in the host. Persistent infection of cattle with noncytopathic BVDV apparently predisposes the animal to clinical, often fatal, mucosal disease after exposure to a cytopathic isolate. It is the overall objective of this proposal to define differences at the molecular level which are responsible for these distinguishing biological properties.

Approach: The structural relationships of the proteins derived from disease producing pairs of cytopathic and noncytopathic BVDV will be determined. Oligonucleotides which may be unique for cytopathic and unique for noncytopathic and those which are conserved for all isolates of BVDV will be identified and sequenced. An oligonucleotide map of the genomes of cytopathic and noncytopathic BVDV will be constructed. The nucleotide sequences which differentiate cytopathic and noncytopathic isolates will be determined.

Progress: 88/01 to 88/12. We have developed a technique to compare the genomes of BVDV strains through fingerprint analyses. The purified RNA is cut with T1 RNase and the resulting fragments are labelled with 32P-pCp. T1 RNase fingerprint analyses are being completed for several strains of BVDV; including Illinois noncytopathic; cytopathic SSD; cytopathic and noncytopathic TGA, and NADL. The fingerprints have been completed and we are in the process of interpreting the data. The preliminary results of the two TGA strains indicate that the cytopathic and noncytopathic

strains isolated from the same animal do not differ genetically since this pair are very similar. The cytopathic SSD is genetically also very close to the TGA whereas the Illinois isolate differs considerably. We are in the process of examining the second strain of the SSD and Illinois pairs to determine the relatedness of several pairs of cytopathic and noneytopathic strains isolated from the same animal. If the pattern is similar to the TGA pairs, it would indicate that the appearance of cytopathic strains in persistently infected animals is the result of a switch of portion of the noncytopathic strain to a cytopathic form. Since in tissue culture these strains retain their characteristic cytopathology, it would seem that such a switch is directed, is a genetically committed situation and is driven by factors not present in cell culture.

Publications: 88/01 to 88/12
NO PUBLICATIONS REPORTED THIS PERIOD.

30.050 CRIS0093813
CLONING AND EXPRESSION OF PROTECTIVE IMMUNOGEN GENES OF BRUCELLA ABORTUS

FICHT T A; ADAMS L C; SOWA B A; Veterinary Microbiology & Parasitology; Texas A&M University, College Station, **TEXAS** 77843.
Proj. No.: TEX06781 Project Type: SPECIAL GRANT
Agency ID: CSRS Period: 01 SEP 84 to 31 DEC 87

Objectives: To isolate the genes encoding outer membrance group 2 and group 3 porius of Brucella abortus. To clone the genes and compare the amino acid sequence of outer membrane group 2 and group 3 porius of virolent and attenuated strains of B. abortus. To establish protective immunity in laboratory animals and cattle by vaccination with recombinant surface immunogeus of B. abortus.

Approach: B. abortus surface immunogeus (group 2 and group 3 perius) will be expressed in E. coli as fusion products of B-galactosidasc. This will be accomplished by the use of the bacteriophage lambda expression vector (lambda gt11).

Progress: 84/09 to 87/12. Brucella abortus DNA (S19 and S2308 fragments of between 300 and 1500 bp) has been successfully cloned and expressed in the lambda gt11 vector system. Recombinants expressing portions of five outer membrane proteins have been purified to homogeniety via selection utilizing antisera raised agaist SDS-PAGE purified proteins prepared from cellular envelopes of a rough mutant of B. abortus. Fusion products of the major outer membrane proteins synthesized under the control of the E. coli lac PO were used to vaccine trials in cattle. Fusion products were purified utilizing an anti-beta-galactosidase column. Twenty-five head of cattle were vaccinated with 3.6 mg each of a cocktail of fusion products in two doses over sixty days representing 30 mu g of brucella antigen. No protection was observed in cattle vaccinated above that experienced by cattle vaccinated with the adjuvant control alone. However, genetic characterization of the recombinants via restriction mapping and DNA sequence analysis has indicated that only a select portion of each gene was fused to beta-galactosidase in the recombinants isolated. As a result, the cattle received a very limited repertoire of outer membrane protein antigens. This may represent a limitation in the lambda gt11 system with regard to the cloning of integral membrane proteins. The B.

Publications: 84/09 to 87/12
FICHT, T.A., BEARDEN, S.W., SOWA, B.A. and ADAMS, L.G. Multiple Copies of a Gene Encoding a Major Outer Membrane Protein of Brucella abortus are Clustered within a Four Kilobase Stretch of Genomic DNA. Infect. and Immun. Submitted for pub.
FICHT, T.A., BEARDEN, S.W. and SOWA, B.A. 1987. DNA sequence of the omp II operon of Brucella abortus. Manuscript in preparation.
FICHT, T.A. and BEARDEN, S.W. 1987. Conservation of the omp II operon in the family Brucellae. Manuscript in preparation.

30.051 0069460
BOVINE BRUCELLOSIS: DIAGNOSIS, VACCINATION IMMUNITY, PATHOGENESIS & EPIDEMIOLOGY

ADAMS L G; CRAWFORD R P; FICHT T A; Veterinary Pathology; Texas A&M University, College Station, **TEXAS** 77843.
Proj. No.: TEX06194 Project Type: HATCH
Agency ID: CSRS Period: 07 MAR 89 to 28 FEB 94

Objectives: (1) To develop and evaluate inactivated and live brucellosis vaccines that do not stimulate antibodies cross-reactive in serologic test, (2) to develop differential and diffinitive assays to diagnose vaccinated or field strain infected cattle, (3) to delineate the genetic basis of the mechanisms of disease pathogenesis and natural resistance, and (4) to identify epidemiologic factors contributing to transmission of Brucella abortus.

Approach: Improved brucellosis vaccines will be approached by: (a) purifying protective immunogens, (b) structural characterization and amino acid sequence determination of immunogens, (c) cloning genes encoding immunogens, and either (d) deleting genes encoding for virulent factors or dominant antigens or inserting protective immunogen genes into expression vectors. Improved differential diagnostics will be approached by (a) isolating strain-specific antigens, (b) developing strain-specific monoclonal antibodies, (c) developing Brucella spp. DNA hybridization probes, (d) or application of monoclonal antibodies in antigen capture ELISA procedures. The mechanisms of disease pathogenesis and natural disease resistance will be approached by: (a) establishing the heritability of disease resistance in cattle, (b) studying B. abortus adhesins for mucosal receptors (c) identifying the mode of entry, invasion and survival of B. abortus and (d) delineating the role of bovine T-lymphocytes in protective immunity.

Progress: 88/01 to 88/12. The inability to diagnostically differentiate between vaccinated and field infected cattle is the major shortcoming of the current USDA approved strain 19 vaccine. We have isolated carbohydrate and protein components of Brucella abortus cell wall and cloned segments of the genes that encode principal protein components of the cell for evaluation as brucellosis vaccines. We recently demonstrated that our killed subunit cell envelope vaccine from a Brucella abortus rough mutant protected 50% of pregnant cows from brucellosis without causing false positives on USDA diagnostic tests. This vaccine is designed to eliminate the problems of false positive reactions and human infections associated with Strain 19 vaccine. Monoclonal antibodies against carbohydrate and protein antigens of Brucella abortus were pyoduced and used in competitive enzyme-linked immunosorbent assay technologies for detection of antigens and differentiating antibodies stimulated by field strain vs Strain 19 vaccine. Trransposon mutagenesis of B. abortus is being used to generate mutants as vaccine candidates which are being evaluated to confirm their lack of reversion to virulence and their ability to induce protective immunity in small ruminants priior to evaluation in cattle.

Publications: 88/01 to 88/12

OVERHOLT, K. TEMPLETON, J.W. and ADAMS, L.G. Standardization of ELISA Calibration Curves the Biomek trademark 1000 Workstation. Beckman Instruments Biomek Bulletin, pp. 1-2, January 1988.

CRAWFORD, R.P., ADAMS, L.G. and RICHARDSON, B.E. Correlation of field strain exposure and brucellosis incidence in 6 beef herds vaccinated with strain 19. J. Am. Vet. Med. Assoc., 5:191-196, 1988.

BARRIOS, D.R., KRAMER, D.C., BESSOUDO, E. and ADAMS, L.G. Embryo collection and bacteriology in Brucella abortus culture-postive superovulated cows. Theriogenology, 29:(2)353-361, 1988.

CRAWFORD, R.P., ADAMS, L.G., and CHILDERS, A.B. Value of Serologic Reactions at 2 Months Following Strain 19 Vaccination of Cattle Herds with Brucellosis. Prev. Vet. Med. 5:(4)275-280, 1988.

CRAWFORD, R.P., SANDERS, R.B. and ADAMS. L. G. Early Removal of Cows with Brucellosis and the Effect in Strain 19 Vaccinated Cattle Herds. Prev. Vet. Med., 5:191-195, 1988.

DAVIS, D.S., HECK, H.C., WILLIAMS, J.D., SIMPSON, T.R. and ADAMS, L.G. Interspecific transmission of Brucella abortus from experimentally infected coyotes (Canis latrans) to parturient Bos taurus heifers. J. Wildl. Dis. 24(3):533-537, 1988.

HARMON, B.G., ADAM, L.G. and FREY, M. Survival of rough and smooth strains of.

30.052 CRIS0089050

MOLECULAR GENETICS OF INTERFERON AND RESISTANCE TO BOVINE RESPIRATORY DISEASE

WOMACK J E; Veterinary Pathology; Texas A&M University, College Station, **TEXAS** 77843.

Proj. No.: TEX06644 Project Type: ANIMAL HEALTH
Agency ID: CSRS Period: 23 NOV 82 to 30 SEP 85

Objectives: Clone bovine interferon genes, to determine their chromosomal location in the cattle genome, and establish their relationship to respiratory disease by the use of restriction endonuclease polymorphisms.

Approach: Bovine interferon genes will be cloned in cDNA plasmids transcribed from mRNA of leucocytes induced to synthesize alpha interferons. Transformed bacterial clones will be selected by hybridization with an interferon-specific synthetic oligonucleotide. DNA will be collected from disease resistant and susceptible cattle, digested with restriction endonucleases, separated by electrophoresis and probed with cloned interferon sequences. Polymorphism will be used to map interferon genes and identify specific relationships of genetic diversity of interferons to respiratory disease.

Progress: 82/11 to 85/12. The objectives of this research are to utilize somatic cell genetic methods to generate a gene map of the cow and to expand this map by isozyme, DNA fragment, and karyotypic analysis. In addition, we are seeking restriction fragment length polymorphisms in cattle using cloned probes from genes of known physiological significance. These polymorphisms will be studies for association with health and productivity in several collaborative studies involving large numbers of cattle. Several important gene families, i.e., interferons, MHC, and growth hormone, have been targeted for cloning, restriction mapping,and genomic mapping. We have generated a gene map of the cow consisting of 36 genes assigned to 23 synthenic groups. Several linkage homologies with mice and humans have been identified, suggesting that large segments of some chromosomes have been conserved in the three mammalian groups. Thus, the cattle homologues of a large number of genes that have been mapped in humans and mice can be presumed to lie in the conserved segment of the cattle chromosome.

Publications: 82/11 to 85/12

WOMACK, J.E. and MOLL, Y.D. A gene map of the cow: conservation of linkage with mouse and man. J. Heredity, in press.

OBRIEN, S.J., SEUANEZ, H.N. and WOMACK, J.E. On the evolution of genome organization in mammals. Evol. Biol. 17, in press.

30.053 CRIS0095472

MOLECULAR GENETICS OF CATTLE

WOMACK J E; Veterinary Pathology; Texas A&M University, College Station, **TEXAS** 77843.

Proj. No.: TEX06718 Project Type: HATCH
Agency ID: CSRS Period: 25 APR 85 to 31 MAR 90

Objectives: To generate a genetic map of cattle chromosomes. To identify restriction fragment length polymorphisms associated with cattle health and productivity. To clone cattle genes for gene injection experiments.

Approach: To combine somatic-cell genetics and recombinant DNA technologies to study the organization of the cattle genome at the molecular level. DNA polymorphisms associated with health and productivity will be identified as gene markers for effective breeding programs. Specific genes useful for improvement of cattle by gene transfer protocols will be identified, isolated, and cloned by molecular genetic technology.

Progress: 88/01 to 88/12. The objectives of this project are to utilize somatic cell genetic methods to generate a gene map of the cow and to expand this map by isozyme, DNA fragment, and karyotypic analysis. Restriction fragment length polymorphisms are being discovered that will permit the combination of recombinant mapping in meiosis with synteny mapping is somatic cells. The ultimate objective is to utilize the map to define markers of disease resistance and productivity. We have now mapped over 100 genes to 26 cow chromosomes. A total of 12 DNA polymorphisms have been discovered, including genes for osteonectin, gamma crystallin 21-steroid hydroxylase, beta hemoglobin, and parathyroid hormone (no reported previously). Each of these has been ddded to the synteny map by analysis of hybrid somatic cells.

Publications: 88/01 to 88/12

WOMACK, J.E.: Genetic engineering in agriculture: animal genetics and development. Trends in Genetics 3:65-68, 1987.

WOMACK, J.E. A gene map of the cow. Genetic Maps 4:499-501, 1987.

ADKISON, L.R., LEUNG, D.W., and WOMACK, J.E.: Somatic cell mapping and restriction fragment analysis of bovine alpha and beta interferon gene families. Cytogenet. and Cell Genet. 1987.

WOMACK, J.E.: Comparative gene maping: A valuable new tool for mammalian developmental studies. Developmental Genetics 8:281-293, 1987.

WOMACK, J.E.: Molecular cytogenetics of cattle: a genomic approach to disease resistance and productivity. J. Dairy Sci. 71:111601123, 1988.

MCAVIN, J.C., PATTERSON, D., and WOMACK, J.E.: Mapping the bovine PRGS and PAIS genes in hybrid somatic cells: syntenic conservation with human chromosome 21. Biochem. Genet. 26:9-18, 1988.

SKOW, L.C. WOMACK, J.E., PETRASH, J.M. and MILLER, W.L.: Synteny mapping of bovine genes for 21 steroid hydroxylase, alpha A-crystallin and class I bovine leucocyte antigen (BoLA) in cattle. DNA 7:143-149, 1988.

30.054 CRIS0097959

MAPPING OF KEY CATTLE GENES INVOLVED IN RESISTANCE TO DISEASE

WOMACK J E; Veterinary Pathology; Texas A&M University, College Station, **TEXAS** 77843.

Proj. No.: TEX06834 Project Type: ANIMAL HEALTH Agency ID: CSRS Period: 14 FEB 86 to 30 SEP 88

Objectives: To genetically map key cattle genes involved in resistance to disease. Specific target genes include the MHC, P-450s, and interferons.

Approach: Panels of hybrid somatic cells segregating cattle chromosomes will be screened with cloned DNA probes. Genes will be mapped by relative concordancy of segregation with previously mapped enzyme coding marker genes.

Progress: 86/01 to 88/09. The objectives of this project were to utilize somatic cell genetic methods to generate a gene map of the cow and to expand this map by isozyme, DNA fragment, and karotypic analysis. Restriction fragment length polymorphisms are being discovered that will permit the combination of recombinant mapping in meiosis with synteny mapping somatic cells. The ultimate objective is to utilize the map to define markers of disease resistance and productivity. We have now mapped over 100 genes to 26 cows chromosomes. A total of 12 DNA polymorphisms have been discovered, including genes for osteonectin, gamma crystallin, and 21-steroid hydroxylase, fibronectin, growth hormone, parathyroid hormone, beta hemoglobin and the three families of interferons. Each of these has been added to the synteny map by analysis of hybrid somatic cells or by in situ hybridization to chromosomes. The polymorphisms discovered have been integrated into a variety of experiments to follow the segregation of host-resistance to pathogens.

Publications: 86/01 to 88/09

WOMACK, J.E. Genetic engineering in agriculture: animal genetics and development. Trends in Genetics 3:65-68, 1987.

WOMACK, J.E. A gene map of the cow. Genetic Maps 4:499-501, 1987.

ADKISON, L.R., LEUNG, D.W. and WOMACK, J.E. Somatic cell m apping and restriction fragment analysis of bovine alpha and beta interferon gene families. Cytogenet. and Cell Genet. 47:62-65, 1988.

WOMACK, J.E. Comparative gene mapping: A valuable new tool for mammalian developmental studies. Developmental Genetics 8:281-293, 1987.

WOMACK, J.E. Molecular cytogenetics of cattle: a genomic approach to disease resistance and productivity. J. Dairy Sci. 71:1116-1123, 1988.

McAvin, J.C., PATTERSON, D. and WOMACK, J.E. Mapping the bovine PRGS and PAIS genes in hybrid somatic cells: syntenic conservation with human chromosome 21. Biochem. Genet. 26:9-18, 1988.

SKOW, L.C., WOMACK, J.E., PETRASH, J.M. and MILLER, W.L. Synteny mapping of bovine genes for 21 steroid hydroxylase, alpha A-crystallin and class 1 bovine leucocyte

antigen (BoLA) in cattle. DNA 7:143-149, 1988.

30.055 CRIS0099941
GENE MAPPING IN CATTLE BY IN SITU HYBRIDIZATION OF G-BANDED CHROMOSOMES

WOMACK J E; Veterinary Pathology; Texas A&M University, College Station, **TEXAS** 77843.
Proj. No.: TEX06867 Project Type: CRGO
Agency ID: CRGO Period: 01 SEP 86 to 31 AUG 89

Objectives: PROJECT 8601309. To map bovine casein, growth hormone, BOLA, 21-OH, and interferon genes to chromosomes. To map these bovine genes to specific chromosomal sites. To develop probes for the in situ mapping of other bovine genes.

Approach: To hybridize radio-labeled probes to cattle chromosomes in situ in order to determine the precise chromosomal location of physiologically significant genes and gene families.

Progress: 88/01 to 88/12. The objectives of this proposal are to utilize in situ hybridization of G-banded chromosomes to physically map genes that are being genetically mapped by other methods. These include interferons, caseins, and several x-linked genes. We have developed methods to stain cattle chromosomes, both by G- and Q- banding, in conjunction with hybridization to labeled probes and autoradiography. Phosphoglycerate kinase-1 clotting factor IX, and the region homologous to human Duchenne muscular dystrophy have been localized on the X chromosome. Probes of high specificity for interferon, fibronectin, gamma crystallin, and caseins have been developed. By using oligolabeling of probes rather than nick-translation, we have been able to use H nucleotides rather than I, and significantly reduce a potential health hazard in the laboratory. The fibronectin and gamma crystallin genes have been successfully localized to chromosome 8, region 1.1 to 1.8. Homologous segments of this chromosome have been identified in mouse and man.

Publications: 88/01 to 88/12

WOMACK, J.E. Genetic engineering in agriculture: animal genetics and development. Trends in Genetics 3:65-68, 1987.
WOMACK, J.E. A gene map of the cow. Genetic Maps 4:449-501, 1987.
SKOW, L.C., ADKISON, L., WOMACK, J.E., BEAMER, W.G. and TAYLOR, B.A. Mapping of mouse fibronectin gene (Fn-1) to chromosome 1: conservation of the Idh-1-Cryg-Fn-1 synteny group in mammals. Genomics 1:283-286, 1987.
ADKISON, L.R., LEUNG, D.W. and WOMACK, J.E. Somatic cell mapping and restriction fragment analysis of bovine alpha and beta interferon gene familes. Cytogent. and Cell Genet. 47:62-65, 1988.
WOMACK, J.E. Molecular cytogenetics of cattle: a genomic approach to disease resistance and productivity. J. Dairy Sci. 71:1116-1123, 1988.
ADKISON, L.R., SKOW, L.C., THOMAS, T.L., PETRASH, M. and WOMACK, J.E. Somatic cell mapping and restriction fragment analysis of bovine genes for fibronectin and gamma crystallin. Cytogenet. and Cell Genet. 47:155-159, 1988.
HALLERMAN, E.M., THEILMANN, J.L., BECKMAN, J.S., SOLLER, M. and WOMACK, J.E. Mapping of bovine prolactin and rhodopsin genes in hybrid somatic cells. Animal Genet. 19:123-131, 1988.

30.056 CRIS0090738
MOLECULAR GENETICS OF INTERFERONS AND RESISTANCE TO BOVINE RESPIRATORY DISEASE

WOMACK J E; CUMMINS J M; Veterinary Pathology; Texas A&M University, College Station, **TEXAS** 77843.
Proj. No.: TEX06783 Project Type: SPECIAL GRANT
Agency ID: CSRS Period: 01 SEP 83 to 28 FEB 86

Objectives: To identify and map cattle genes involved in interferon production and sensitivity and to map their chromosomal position relative to other gene markers.

Approach: Hybridization of somatic cells (cattle-rodent) to produce hybrid clones segregating cattle chromosomes. Cattle genes will be identified by isozyme technology and molecular probing of restriction endonuclease fragments. Karyology will be done by G-banding; concordant loss and retention of cattle genes will identify syntenic groups that will then be assigned to specific chromosomes by karyotypic concordancy. Cloned probes of interferon genes will be used to identify the number of bovine interferon genes, their location in the genome, and their relationship to specific pathogens of the respiratory disease complex.

Progress: 83/09 to 85/12. The objectives of this research are to utilize somatic cell genetic methods to generate a gene map of the cow and to expand this map by isozyme, DNA fragment, and karyotypic analysis. In addition, we are seeking restriction fragment length polymorphisms in cattle using cloned probes from genes of known physiological significance. These polymorphisms will be studied for association with health and productivity in several collaborative studies involving large numbers of cattle. Several important gene families, i.e., interferons, MHC, and growth hormone, have been targeted for cloning, restriction mapping, and genomic mapping. We have generated a gene map of the cow consisting of 36 genes assigned to 23 syntenic groups. Several linkage homologies with mice and humans have been identified, suggesting that large segments of some chromosomes have been conserved in the three mammalian groups. Thus, the cattle homologues of a large number of genes that have been mapped in humans and mice can be presumed to lie in the conserved segment of the cattle chromosome.

Publications: 83/09 to 85/12

WOMACK, J.E. and MOLL, Y.D. A gene map of the cow: conservation of linkage with mouse and man. J. Heredity, in press.
O'BRIEN, S.J., SUANEZ, H.N. and WOMACK, J.E. On the evolution of genome organization in mammals. Evol. Biol. 17, in press.

30.057 CRIS0133007
MOLECULAR GENETICS OF INTERFERONS & RESISTANCE TO BOVINE RESPIRATORY DISEASE

WOMACK J E; HUTCHESON D P; Veterinary Pathology; Texas A&M University, College Station, **TEXAS** 77843.
Proj. No.: TEXO6914 Project Type: SPECIAL GRANT
Agency ID: CSRS Period: 01 SEP 87 to 31 AUG 90

Objectives: To test the putative correlation of restriction fragment lenght polymorphisms (RFLPs) in interferon genes with morbidity due to IBR virus. To test the correlation of RFLPs in interferon genes with bovine respiratory disease. To determine the relationships of interferon RFLPs with interferon synthesis. To further characterize the extent and nature of natural variation in interferon genes in breeding populations of cattle.

Approach: Experimental feedlot facilities and challenge with IBR virus will be used to assess the genetic variation in cattle to respiratory disease and the shipping fever syndrome. The relationship of previously identified variation in interferon genes will be compared to inate resistance to respiratory disease and the ability of different cattle to synthesize interferons.

Progress: 88/01 to 88/12. We have collected blood and extracted DNA from over 50 animals that have been exposed to IBR virus. Testicular biopsy has been used to initiate tissue cultures that can eventually be assayed for response to interferon inducers. Time of electrophoresis has been increased to improve separation of DNA fragments representative of the critical IFNB alleles. Interferon gene polymorphisms have been identified which permit the analysis of genomic constitution relative to innate resistance to IBR virus. A significant association has been discovered between a beta interferon allele and susceptibility to IBR virus. This DNA marker holds promise for use in the selective breeding of resistant animals. Beta interferons appear to exist as a hypervariable family of genes in cattle, with at least one locus giving the appearance of a variable number of tandem repeats (VNTR).

Publications: 88/01 to 88/12
WOMACK, J.E. Molecular cytogenetics of cattle. A genomic approach to disease resistance and productivity. J. Dairy Sci. 71:1116-1123, 1988.
SKOW, L.C., ADKISON, L., WOMACK, J.E., BEAMER, W.G. and TAYLOR, B.A. Mapping of mouse fibronectin gene (Fn-1) to chromosome 1: conservation of the Idh-1-Cry-Fn-1 synteny group in mammals. Genomics 1:283-286, 1987.
ADKISON, L.R., LEUNG, D.W. and WOMACK, J.E. Somatic cell mapping and restriction fragment analysis of bovine alpha and beta interferon gene families. Cytogenet. and Cell Genet. 47:62-65, 1988.
MCAVIN, J.D., PATTERSON, D. and WOMACK, J.E. Mapping the bovine PRGS and PAIS genes in hybrid somatic cells: syntenic conservation with human chromosome 21. Biochem. Genet. 26:9-18, 1988.
SKOW, L.C., WOMACK, J.E., PETRASH, M. and MILLER, W.L. Synteny mapping of bovine genes for 21 steroid hydroxylase, alpha A-crystallin and class I bovine leucocyte antigen (BoLA) in cattle. DNA 7:143-149, 1988.
ADKISON, L.R., SKOW, L.C., THOMAS, T.L., PETRASH, M. and WOMACK, J.E. Somatic cell mapping and restriction fragment analysis of bovine genes for fibronectin and gamma crystallin. Cytogenet. and Cell Genet. 47:155-159, 1988.
HALLERMAN, E.M., THEILMANN, J.L., BECKMANN, J.S., SOLLER, M. and WOMACK, J.E. Mapping of bovine prolactin and rhodopsin genes in hybrid somatic cells. Animal Genet. 19:123-131, 1988.

30.058 CRIS0096961
MOLECULAR AND SOMATIC CELL GENETICS: MAPPING THE CATTLE GENOME

WOMACK J E; SKOW L C; BAKER J F; Veterinary Pathology; Texas A&M University, College Station, **TEXAS** 77843.
Proj. No.: TEXO6795 Project Type: CRGO
Agency ID: CRGO Period: 01 SEP 85 to 31 AUG 88

Objectives: Proj 8502086. To generate a gene map of the cow including genes of physiological significance, to determine the extent and nature of variation of these genes in breeding populations, and to determine the molecular organization and genetic variability of key multigene families.

Approach: We will utilize somatic cell genetic methods to map the cattle genome, assigning enzymes and DNA fragments to specific cattle chromosomes. The same probes used to map DNA fragments will be used to seek restriction fragment length polymorphisms (RFLPs) in cattle populations and to determine the complexity and variability of multi-gene families. The discovery and mapping of new genetic variation will provide a sound scientific basis for genetic improvement, either by conventional breeding or by emerging gene transfer technology.

Progress: 85/09 to 88/08. DNA from over 700 cattle representing crosses of eight breeds has been prepared and analyzed. Up to 100 samples have been digested with at least six restriction enzymes and examined for restriction fragment length polymorphisms (RFLPs) with molecular probes for growth hormone, alpha-crystallin, 21-hydroxylase, and fibronectin, prolactin, beta hemoglobin, and parathyroid hormone. Polymorphism has been found with each probe. The same probes have been used to examine DNA from cattle-hamster

hybrid somatic cells. All of the genes have been mapped to bovine syntenic groups, bringing the total number of mapped genes in the cow to over 100.

Publications: 85/09 to 88/08

WOMACK, J.E. and MOLL, Y.D.: A gene map of the cow: conservation of linkage with mouse and man. J. Heredity 77:2-7, 1986.
WOMACK, J.E.: Genetic engineering in agriculture: animal genetics and development. Trends in Genetics 3:65-68, 1987.
WOMACK, J.E.: A gene map of the cow. Genetic Maps 4:499-501. 1987.
WOMACK, J.E.: Comparative gene mapping: A valuable new tool for mammalian developmental studies. Developmental Genet. 8:281-293, 1987.
ADKINSON, L.R., LEUNG, D.W. and WOMACK, J.E.: Somatic cell mapping and restriction fragment analysis of bovine alpha and beta interferon gene families.
Cytogenet. and Cell Genet. 47:62-65, 1988.
WOMACK, J.E.: Molecular cytogenetics of cattle: a genomic approach to disease resistance and productivity. J. Dairy Sci. 71:1116-1123, 1988.
MCAVIN, J.C., PATTERSON, D. and WOMACK, J.E.: Mapping the bovone PRGS and PAIS genes in hybrid somatic cells: syntenic conservation with human chromosome 21.
Biochem, Genet, 26:9-18, 1988.

30.059 CRIS0097254

GENES CODING FOR INSULIN IMMUNOREACTIVITY IN FETAL BOVINE PANCREAS DEVELOPMENT

FRAZIER M L; Anderson Hospital & Tumor Inst; University of Texas, Houston, **TEXAS** 77030.
Proj. No.: TEXR-8502216 Project Type: CRGO
Agency ID: CRGO Period: 15 SEP 85 to 31 MAR 88

Objectives: Proj 8502216. We have obtained evidence that two distinct species of mRNA coding for insulin immunoreactivity (IRI) are produced by the fetal bovine pancreas. The overall objective of this proposal is to characterize the genes coding for IRI and their expression during fetal pancreas development.

Approach: A specific cDNA probe will be identified for each of the two mRNAs coding for IRI and the nucleotide sequence will be eluciated. A developmental study will be conducted to determine if the two mRNAs coding for IRI are differentially expressed. Genomic clones for the bovine insulin gene will be isolated to determine the number of bovine insulin genes and the corresponding nucleotide sequences. If two mature forms of insulin are predicted, the pancreas content of insulin will be analyzed to determine if there is more than one form of mature insulin, and the expression of the different insulins will be determined during gestation.

Progress: 85/09 to 88/03. We have obtained evidence that two distinct species of mRNA coding for insulin immunoreactivity (IRI) are produced by the fetal bovine pancreas. The overall objective of this project was to characterize the genes coding for IRI and their expression during fetal bovine pancreas development. We prepared a cDNA library from poly(A+)RNA isolated from fetal bovine pancreas. Bacterial colonies were screened for sequences homologous to a rat preproinsulin I cDNA probe. Northern blot analyses revealed that seven of the clones hybridized to a single RNA species of approximately 400 nucleotides. Sequence analysis of one of the clones (pbI2885) revealed the entire structural region of bovine preproinsulin mRNA including a 72 nucleotide region encoding a signal peptide enriched in hydrophobic residues. We have also cloned and sequenced a cDNA clone coding for bovine preprosomatostatin, which will be valuable for comparative studies on the ontogeny of islet cell specific genes. When 10 ug of bovine genomic DNA were digested with restriction endonuclease Bam HI, subjected to Southern blot analysis, and hybridized with P insert isolated from pbI2885, six fragments were identified (8.6, 5.7, 4.7, 3.3, 2.2, and 1.9 kb in length) containing insulin homologous fragments. When the DNA was digested with MboI instead, five bands were identified (4.4, 3.9, 2.2, 1.8, and 1.3 kb in length) containing insulin homologous sequences.

Publications: 85/09 to 88/03

DAGOSTINO, J.B., YOUNES, M.A., WHITE, J.W., BESCH, P.K., FIELD, J.B. and FRAZIER, M.L. 1987. Cloning and nucleotide sequence analysis of complementary deoxyribonucleic acid for bovine preproinsulin. Molecular Endocrinology. 1:327-331.
SU, C.J., WHITE, J.W., LI, W.H., LUO, C.C., FRAZIER, M.L., SAUNDERS, G.F. and CHAN, L. 1988. Structure and evolution of somatostatin genes. Molecular Endocrinology. 1:209-216.

30.060 CRIS0093513

MOLECULAR CLONING AND SYNTHETIC VACCINE STUDIES OF BLUETONGUE VIRUS

LI J K K; Biology; Utah State University, Logan, **UTAH** 84322.
Proj. No.: UTA00537 Project Type: ANIMAL HEALTH
Agency ID: CSRS Period: 01 JUL 84 to 30 JUN 89

Objectives: The major objectives are: to understand the molecular processes of Bluetongue Virus (BTV) replication morphogenesis, and transmission. To investigate the functional domains of BTV polypeptides. To determine the host immunological responses to BTV infection. To develop more effective vaccines against BTV.

Approach: Most of the genes of different BTV serotypes will be cloned by recombinant DNA techniques. The nucleotide sequences of relevant genes will be determined. The amino acid sequences of the gene products will be deduced from DNA sequences. After hydrophobicity and hydrophilicity analyses, synthetic oligopeptides which represent relevant BTV polypeptide subsets, will be synthesized. These synthetic peptides which are potential vaccines and their monospecific antiserum will be used for biological,

biochemical, immunological and vaccine studies. These studies will also be carried out with BTV proteins and their peptide fragments obtained by biochemical means and column chromatography.

Progress: 88/01 to 88/12. Several genes of BTV serotypes 10, 11, 13 and 17 have been partially cloned. The genomic sequence of S1 of BTV-13 which codes for the major core protein VP7 has been completely sequenced by difference sequencing methods. Sequence homology between S1 gene of BTV 10 and 13 is about 75%. However, homology of amino acid sequence is over 90%. Genetic relatedness of the five US BTV serotypes has also been further investigated by both enzymatic and chemical peptide mapping. The results further substantiate our previous RNA/RNA hybridization data. Selective purification of BTV ds-RNA genome and viral proteins has been achieved by a rapid SDS/KC1 precipitation method. Both polyclonal and monoclonal antibodies against VP5 and 7 have been produced. These antisera are very useful in immunoblots and diagnostic ELISA for BTV. The potential uses of this non-infectious SDS/KC1 precipitated BTV viral protein as a vaccine for BTV will be further examined. Using BTV ds-RNA's and viral proteins isolated by the SDS/KC1 method as well as the antibodies produced, a diagnostic method for BTV has been developed. A rapid alkaline Northern transfer method for BTV single-stranded mRNAs has also been developed. This method produces two identical blots within one hour and will be useful for the viral morphogenesis study.

Publications: 88/01 to 88/12

KOWALIK, T.F. and LI, J.K.-K. 1988. Cognate gene analysis and genetic relatedness of Bluetongue viruses by RNA/RNA hybridization (Osburn, B. and Roy, P., eds.). pp. 45-52, Univ. of CA press, Davis, CA.

PARKER, B. and LI, J.K.-K. 1988. A method to produce six alkaline Northern blots of viral-RNA within one hour. Biotechniques, 6:22-23.

PARKER, B. and LI, J.K.-K. 1988. Alkaline Northern blotting of ds-RNA to Zeta-Probe membranes. Molecular Biology Reports 4:3-4.

LI, J.K.-K., JOHNSON, T. and PARKER, B. 1988. Multiple uses of sterile liquepipets in Southern, Northern and Western blots (Anal. Biochemistry, in press).

PARKER, B. 1988. Cloning and expression of an EBV-associated DNA polymerase. Ph.D. Thesis, USU, Logan, UT. 54 pp.

LEU, S.J. 1988. Characterization of Wolbachia postica which causes hybrid incompatibility in alfalfa weevils. Master Thesis. USU, Logan, UT. 45 pp.

HWANG, G.Y. 1988. Purification and characterization of a chemically-induced EBV-associated deoxyribonuclease. M.S. Thesis, USU, Logan, UT. 42 pp.

30.061 CRIS0099954

MOLECULAR CLONING, GENOME ANALYSIS AND GENE PROBES OF BLUETONGUE VIRUSES

LI J K K; Business & Administration; Utah State University, Logan, **UTAH** 84322.

Proj. No.: UTA00605 Project Type: CRGO
Agency ID: CRGO Period: 15 SEP 86 to 30 SEP 89

Objectives: PROJECT 8601003. Determination of the group-specific and serotype-specific genes of BTV 2, 10, 11, 13 and 17 by RNA/RNA hybridization; cloning of one of the group-specific genes in these five serotypes and preparation of both isotopic and non-isotopic BTV group-specific gene probes.

Approach: Both the group-specific and serotype-specific genes of BTV 2, 10, 11, 13 and 17 will be identified by both dot-blot and Northern blot hybridization using both isotopic, total genomic ds-RNA and individually isolated BTV RNA segments. The most group-specific genes of these five U.S. BTV serotypes will then be cloned by several methods. The sequences of these clones will be determined by both dideoxy chain termination and chemical sequencing methods. These clones will be used to develop both isotopic and biotinylated gene probes which will be used for potential diagnosis of BTV in blood or semen samples when they are available.

Progress: 88/01 to 88/12. Several genes of BTV serotypes 10, 11, 13 and 17 have been partially cloned. The genomic sequence of S1 of BTV-13 which codes for the major core protein VP7 has been completely sequenced by difference sequencing methods. Sequence homology between S1 gene of BTV 10 and 13 is about 75%. However, homology of amino acid sequence is over 90%. Genetic relatedness of the five US BTV serotypes has also been further investigated by both enzymatic and chemical peptide mapping. The results further substantiate our previous RNA/RNA hybridization data. Selective purification of BTV ds-RNA genome and viral proteins has been achieved by a rapid SDS/KC1 precipitation method. Both polyclonal and monoclonal antibodies against VP5 and 7 have been produced. These antisera are very useful in immunoblots and diagnostic ELISA for BTV. The potential uses of this non-infectious SDS/KC1 precipitated BTV viral protein as a vaccine for BTV will be further examined. Using BTV ds-RNAs and viral proteins isolated by the SDS/KC1 method as well as the antibodies produced, a diagnostic method for BTV has been developed. A rapid alkaline Northern transfer method for BTV single-stranded mRNAs has also been developed. This method produces two identical blots within one hour and will be useful for the viral morphogenesis study.

Publications: 88/01 to 88/12

KOWALIK, T.F. and LI, J.K.-K. 1988. Cognate gene analysis and genetic relatedness of Bluetongue viruses by RNA/RNA hybridization. In: Orbioviruses and Birnaviruses (Osburn, B., Roy, P. eds). pp. 45-52, Univ. of CA, Davis, CA.

PARKER, B. and LI, J.K.-K. 1988. A method to produce six alkaline Northern blots of viral-RNA within one hour. BioTechniques,

6:22-23.
PARKER, B. and LI, J.K.-K. 1988. Alkaline Northern blotting of ds-RNA to Zeta-Probe membranes. Molecular Biology Reports 4:3-4.
LI, J.K.-K., JOHNSON, T. and PARKER, B. 1988. Multiple uses of sterile liquepipets in Southern, Northern and Western blots. Anal. Biochemistry, 174:471-476.

30.062 CRIS0095764
PEPTIDE AGAINST BOVINE HEAT-STABLE ENTEROTOXIN BY IN-VITRO DIRECTED MUTAGENESIS

SRIRANGANATHAN N; BOYLE S; SCHURIG G; College of Vet Medicine; Va-md Regional Coll of Vet Med, Blacksburg, **VIRGINIA** 24061.
Proj. No.: VA-135185 Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 85 to 30 SEP 88

Objectives: To produce a peptide that is non-toxic but would bind to the heat-stable enterotoxin (STa) receptor i.e., cross reacting material (CRM).

Approach: This will be accomplished by in-vitro directed mutagenesis of STa gene by specific amino acid substitution. Then the mutant STa gene will be ligated into appropriate vector and used in the transformation of E. coli. Using antibodies produced against native STa, we will select transformants (with synthetic gene) that produces STa-like material and test these potential CRM in suckling mouse assay for their ability to block the action of native STa.

Progress: 87/01 to 88/09. A Heat-Stable enterotoxin (STa) positive clone (IX) was created by introducing a Taq I fragment containing STa gene from pSLM004 into the multiple cloning site of M13mp18.Synthetic DNA oligomers representing the proposed amino acid substitutions were used as primers in the enzymatic synthesis of the mutant on the single stranded phage DNA. The heteroduplexes thus generated were utilized in the transformation of Su2 suppressor bearing E. coli JM83 to selectively inhibit the replication of the wildtype STa sequence. Once enriched the mutant STa was moved back into JM109. The specific activities of the two such purified mutant peptides were comparable to the specific activity of native peptide. This suggested that the mutations did not greatly alter the biological activity. The third mutant proposed is being produced on a large scale and will be tested for its biological activity in suckling mice. The mutant DNA sequences so far generated appears to be on the flanking regions of the site of directed mutagenesis. Attempts are under way to extend the DNA sequence generated by altering the conditions of enzymatic synthesis. Amino acid sequence will be determined of purified mutant peptides to confirm the mutation.

Publications: 87/01 to 88/09
NO PUBLICATIONS REPORTED THIS PERIOD.

30.063 CRIS0135514
CHARACTERIZATION OF THE BRUCELLA ABORTUS GENOME BY PHYSICAL AND GENETIC TECHNIQUES

BOYLE S M; Vet Medical Experiment Station; Va-md Regional Coll of Vet Med, Blacksburg, **VIRGINIA** 24061.
Proj. No.: VA-137117Project Type: ANIMAL HEALTH
Agency ID: CSRS Period: 01 OCT 88 to 30 SEP 91

Objectives: Generate a physical map of the B. abortus genome utilizing pulse field gel electrophoresis. Generate a transposon map of the B. abortus genome utilizing P1::Tn5 mutagenesis. Construct a macro-restriction map of B. abortus genome by combining the transposon and the physical maps.

Approach: DNA from avirulent and virulent B. abortus strains will be hydrolyzed with restriction enzymes recognizing rare sequences; the digested genomic fragments will be seperated by pulse field get electrophoresis, strains of B. abortus will be infected with P1::Tn5; the DNA from Kn resistant clones will be characterized by pulse field gel electrophoresis. A macro-restriction map will be constructed by comparing the mobility of the DNA fragments from uninfected cells to those infected with P1::Tn5 on a pulse field gel electrophoreogram.

30.064 CRIS0135245
THE NATURE AND CONSEQUENCES OF SUPEROXIDE DISMUTASE EXPRESSION IN BRUCELLA ABORTUS

BOYLE S M; MISRA H; SCHURIG G; Regional College of Vet Med; Virginia Poly Inst, Blacksburg, **VIRGINIA** 24061.
Proj. No.: VA-135257 Project Type: HATCH
Agency ID: CSRS Period: 15 JUL 88 to 30 JUN 91

Objectives: These experiments will allow the identification, isolation and characterization of the superoxide dismutase (sod) genes of Brucella abortus. An assessment will be made of sod genes structure and function as well as of the relationship of sod gene expression to the ability of Brucella to replicate as an intracellular pathogen.

Approach: The sod genes will be cloned by complementation of sod deficient E. coli or screening by hybridization of a genomic library of Brucella DNA with the sodA and sodB genes of E. coli. The sod genes will be characterized by nucleotide sequencing and expression in E. coli mini-cells. The sod genes will be mutagenized either with the transposon Tn5 or by creation of anti-sense sod genes. The ability of sod deficient Brucella to replicate in mice will be used to measure any changes in virulence.

30.065 CRIS0141611
GENETIC ANALYSIS OF VIRULENT AND AVIRULENT BOVINE HERPESVIRUSES

SHEN D T; KNOWLES D P; GORHAM J R; Agricultural Research Service, Pullman, **WASHINGTON** 99164.
Proj. No.: 5348-34000-002-00D
Project Type: INHOUSE
Agency ID: ARS Period: 05 JAN 87 to 05 JAN 92

Objectives: 1) Establish and compare physical and functional genetic maps of BHV-1 strains; 2) Identify regions of divergence among virulent and modified strains; 3) Clone regions containing diverging nucleotide-sequences; 4) Characterize and express the gene-products encoded in these sequences; 5) Prepare diagnostic reagents such as gene-probes and monoclonal antibodies; 6) Use these reagents to study in vitro and in vivo expression of such genes.

Approach: Molecular analysis of the genome structure and gene expression of bovine herpesviruses will be used to: 1) Establish and correlate physical and functional genetic maps of virulent and avirulent BHV-1 strains; 2) PrepareC-DNA from viral transcripts of the "immediate early" and "early" stages of virus-replication; 3) Identify and clone DNA, C-DNA sequences which seemto correlate with expression of virus-virulence; 4) Produce sensitive nucleic acid probes and immunologic reagents with which such DNA sequences or their gene products can be detected; and 5) Evaluate by in vitro and in vivo tests the diagnosatic practicability of such probes and reagents for the detection of specific DNA sequences of gene products.

Progress: 88/01 to 88/12. Fragments of DNA generated by cleavage of viral DNA of BHV-1 strain LA and BHV-4 strain DN599 with the restriction endonuclease Hind III were ligated into plasmids and amplified by replication in E. coli strain DH 5 a. Purified recombinant DNA was labeled enzymatically with biotin by nick-repair--or random-primed repair synthesis, or photochemically by reaction with photobiotin-acetate. Binding of labeled DNA to immobilized target-DNA was detected with a strepto- vidin-alkaline phosphatase detection system. All three labeling techniques produced probes of comparable specificity for homologous sequences in the target DNA; however, random-primed probes gave the strongest signals. Because of the low cost and simplicity we used the photo-labeling method to compare the ability of the recombinant- DNA probes to detect homologous DNA against a background of bovine DNA. The results indicated that both viral DNAs exhibited unique sequences which can be used to identify viral DNA in nucleic hybridization tests.

Publications: 88/01 to 88/12

GUO, W.Z., SHEN, D.T., EVERMANN, J.F., GORHAM, J.R. 1988. Comparison of an enzyme-linked immunosorbent assay and a complement fixation test for the detection of IgG to bovine herpesvirus type 4 (bovine cytomegalovirus). 9th Annual. continued - Western Food Animal Disease Research Conference. Moscow, Idaho. (Abstract).

BURGER, D., SHEN, D.T., GORHAM, J.R. 1988. Detection of Bovine Herpesvirus Type 1 (BHV-1) and Bovine Herpesvirus Type 4 (BHV-4) DNA by Hybridization with Non-radioactive Nucleic Acid Probes. 9th Annual Western Food (continued). (continued) - Animal Disease Research Conference. Moscow, Idaho (abstract).

GUO, W.Z., SHEN, D.T., EVERMANN, J.F., GORHAM, J.R. 1988. Comparison of an enzyme-linked immunosorbent assay and a complement-fixationn test for the strong est signals. Because of the low cost and simplicity we used thephoto-labeling. (5 continued). Am. Journal Vet. Research 49(5) 667-670.

30.066 CRIS0097149
GENETIC ORGANIZATION AND FUNCTION IN THE PRODUCTION OF THE F41 BACTERIAL ADHESIN

MOSELEY S L; Microbiology; University of Washington, Seattle, **WASHINGTON** 98195.
Proj. No.: WNR-8502362 Project Type: CRGO
Agency ID: CRGO Period: 01 SEP 85 to 31 AUG 87

Objectives: Proj 8502362. The proposed research seeks to characterize the genetic basis for the production of E. coli adhesins F41 by enterotoxigenic E. coli. The plasmid or chromosomal location of the gene will be determined, and the number and function of gene products will be analyzed. The genetic relatedness of F41 with other E. coli adhesins will be determined. The nucleotide sequence of the DNA encoding the F41 structural subunit will be determined. A specific hybridization probe for the detection of strains of E. coli which product F41 by colony DNA hybridization will be developed.

Approach: The cloned F41 gene will be physically mapped by restriction analysis. The gene will be localized by analysis of transposon Tn5 insertion mutants. The Tn5 insertion mutants, as well as the wild type cloned gene, will be analyzed for expression of products in an E. coli minicell system. Function of the various products will be determined by phenotypic expression by the insertion mutants of antigen and hemagglutination properties. Genetic relatedness will be studied by Southern blot hybridization of F41-encoding DNA with DNA encoding other adhesins of E. coli. An appropriate restriction fragment hybridizing only to the F41 gene will be developed as an F41 specific hybridization probe.

Progress: 87/01 to 87/08. Genes encoding the production of the F41 bacterial adhesin have been isolated from an enterotoxigenic Escherichia coli strain pathogenic for pigs, and characterized. The determminant was found by Southern blot hybridization to be chromosomal in all F41-producing strains exmined. Four gene products were identified by E. coli maxicell analysis to be associated with F41 production, ad two additional genes were

identified by nucleotide sequence analysis. The genes were physically mapped. The entire DNA region encoding F41 was found to share extensive homology with the K88 determinant of E. coli, with the exception of the regions encoding the fimbrial subunits which were non-homologous. The genetic organization of F41 was very similar to that of K88. Nucleotide sequence analysis of the structural subunit gene of F41 predicted a product with structural similarities to K88 and other E. coli fimbriae. K88 and F41 specific hybridization probes were derived from the structural subunit genes of each determinant. Hybridization probes were used to examine a number of E. coli isolates from animals and humans. Some animal isolates reacting with probes derived from sequences shared by K88 and F41 determinants produced neither K88 nor F41. Several of these strains produced fatal septicemia in newborn colostrum-deprived pigs. The probes also detected human enteroinvasive E. coli isolates, and the homology was localized to the large virulence-associated plasmid of these strains.

Publications: 87/01 to 87/08

MOSELEY, S.L., DOUGAN, G., SCHNEIDER, R.A. and MOON, H.W. 1986. Cloning of chromosomal DNA encoding the F41 adhesin of enterotoxigenic Escherichia coli and genetic homology between adhesins F41 and K88. J. Bacteriol. 167:799-804.

RUNNELS, P.L., MOSELEY, S.L., MOON, H.W. 1987. F41 pili as protective antigens of enterotoxigenic Escherichia coli that produce F41, K99, or both pilus antigens. Infect. Immun. 55:555-558.

30.067 CRIS0096936

DEVELOPMENT OF A RECOMBINANT DNA DERIVED VACCINE AGAINST BOVINE ANAPLASMOSIS

BARBET A F; College of Vet Medicine; Washington State University, Pullman, **WASHINGTON** 99164.
Proj. No.: WNV-1-1757 Project Type: CRGO
Agency ID: CRGO Period: 01 SEP 85 to 31 DEC 85

Objectives: Proj. 8502937. Characterize recombinant clones of E. coli expressing epitopes of Am 105; obtain the sequence of the gene coding for Am 105; localize Am 105 epitopes which induce neutralizing monoclonal antibodies.

Approach: The approach is to use recombinant DNA technology, including the use of monoclonal antibodies, to accomplish the objectives.

30.068 CRIS0096211

A POLYVALENT VACCINIA VIRUS RECOMBINANT VACCINE FOR BLUETONGUE

BREEZE R G; GORHAM J R; College of Vet Medicine; Washington State University, Pullman, **WASHINGTON** 99164.
Proj. No.: WNV-2-2608 Project Type: SPECIAL GRANT
Agency ID: CSRS Period: 01 SEP 85 to 31 AUG 87

Objectives: The objective is to make progress toward the development of a polyvalent vaccine for bluetongue through cloning the gene for the serotype specific protein of BTV-13 and then expressing it in an infectious vaccinia virus recombinant.

Approach: The approach includes: Identification of the gene segment which codes for the serotype specific protein which induces protective immunity for BTV-13; cloning of the gene for this serotype specific protein; construction of an infectious vaccinia virus vector which expresses the immunogenic protein of BTV-13; and demonstration of humoral and cellular immunity and protection against live BTV-13 challenge in mice and sheep vaccinated with this vaccinia vector.

Progress: 86/01 to 86/12. We have made monoclonal antibodies to blue tongue virus serotype 13, and these are presently being characterized. We have also isolated the RNAs of the 10 segments of the virus. Cloning work has not been intiated.

Publications: 86/01 to 86/12

NO PUBLICATIONS REPORTED THIS PERIOD.

30.069 CRIS0093688

MOLECULAR CLONING OF THE CDNA GENE FOR BOVINE INTERLEUKIN-2

MAGNUSON J A; REEVES R; MAGNUSON N S; Office of Research Resources; Washington State University, Pullman, **WASHINGTON** 99164.
Proj. No.: WNV02406 Project Type: SPECIAL GRANT
Agency ID: CSRS Period: 01 SEP 84 to 31 AUG 87

Objectives: The major objective of these studies is to develop a method for the production of large quantities of interleukin-2 (IL-2) for the control of bovine shipping fever.

Approach: cDNA of IL-2 enriched mRNA populations will be synthesized using reverse transcriptase and gene copies will be subsequently incorporated into pBR 322 plasmids. After identification of the functional cDNA gene, it will be isolated from its plasmid vehicle and ligated into an appropriate expression vector. It will then be introduced into an E. coli or yeast host strain for production of large quantities of protein.

Progress: 87/01 to 87/12. A cDNA clone of the bovine interleukin-2 (IL-2) gene was obtained and sequenced. Functional IL-2 was produced by monkey cells transfected with the appropriate gene. We have also obtained functional IL-2 from yeast transfected with a vector containing the appropriate gene. We are processing this IL-2 for future work. We have now obtained the cDNA for the bovine IL-2 receptor (IL-2R) and have it partially sequenced. We are currently finishing the sequence determination and preparing antibody to the receptors.

Publications: 87/01 to 87/12
WEINBERG, D., ENIOT, J., PAETKAU, V., BLEAKLEY, C., MAGNUSON, N., REEVES, R. and MAGNUSON, J. Cloning of the cDNA for the bovine interleukin-2 (IL-2) receptor (bovine "TAC antigen"). Immunology (in press).
MAGNUSON, N.S., SPIES, A.G., NISSEN, M.S., BUCK, C.D., WEINBERG, A.D., BAN, P.J., MAGNUSON, J.A. and REEVES, R. Bovine interleukin-2: Regulatory Mechanisms (in press).

30.070 0141672
CULICOIDES VARIIPENNIS; VECTOR BIOLOGY AND VECTOR COMPETENCE FOR BLUETONGUE VIRUS

WALTON T E; NUNAMAKER R A; HOLBROOK F R; Agricultural Research Service, Laramie, **WYOMING** 82070.
Proj. No.: 5410-34350-001-00D
Project Type: INHOUSE
Agency ID: ARS Period: 01 NOV 86 to 01 NOV 91

Objectives: Investigate variability and systematic status of C. variipennis (CV). Improve procedures for colonization and maintenance of biting midges. Investigate vector-virus-host interactions in BTV cycle. Improve sampling techniques for arthropod vectors. Evaluate seasonal dynamics of the vector-BTV relationship and produce a predictive model. Characterize genetics of CV competence for BTV.

Approach: Electrophoretic, chromatographic (isozyme/hydrocarbon) and EM studies of CVpopulations will be correlated to BTV vector potential. Specific survey techniques will be utilized to evaluate vector-BTV events, correlate with meteorological events and develop a BTV predictive model. Evaluations of vector-virus survey techniques will continue in Wyoming, Colorado and Nebraska. Biologically secure rearing and handling systems for genetically defined CV colonies will be evaluated and a standard manual of techniques prepared. Selected genetic lines of CV will be assayed for oral suscepti- bility to BTV and phenotypic analyses developed using traditional and molecular biologic techniques. Selected lines will be genotypically analyzed for vector competence, biochemical characteristics and morphological characters. Environmental influences on genetic characteris- tics will be lab evaluated.

Progress: 88/01 to 88/12. Cv is the primary US vector of BTV. Wild & colonized adults were marked with RbCl to study flight range. Two natural, genetically distinct US Cv populations were identified isozymically: Cv variipennis in the northeast & Cv sonorensis in the west. Natural Cvs populations show seasonal genetic changes. Established isofemale Cvs lines of known family groups to characterize by infection rates & competence. Cloned Cvs DNA fragments to study genetic diversity. High incubation temperature (26C vs 20C) increased Cvs mortality. Infection with 3 different BTV serotypes caused no differences in Cvs mortality. Examined adult Cv midguts using immunogold-labelled ultrathin cryosections (IGLUC) after infection with BTV-11; BTV was seen on/within erythrocytes & in midgut cells. IGLUC with monoclonal antibody detected BTV in Cv developing oocytes: BTV was not transmitted transovarially to offspring thru 5 gonatrophic cycles, but antigen was detected in yolk bodies & vitelline membrane of oocytes indicating BTV may penetrate the ovarian sheath. Each of the paired Cv salivary glands consists of 5 lobes attached at the gland's base. Glandular cells had abundant endoplasmic reticulum, mitochondria & microtubules; a basal lamina borders the epithelial cells lining the salivary duct. IGLUC detected BTV antigen in cytoplasm & plasma membrane of salivary gland acinar cells & detected mature virions & antigen extracellularly & in cisternae of vacuoles & endoplasmic reticulum.

Publications: 88/01 to 88/12
NUNAMAKER, R.A., WICK, B.C. and NUNAMAKER, C.E. 1988. Salivary glands of female Culicoides variipennis (Diptera:Ceratopogonidae): Morphologic changes associated with maturation and blood-feeding. Proc. International Congr. Entomol. 28:90.
NUNAMAKER, R.A., WICK, B.C. and NUNAMAKER, C.E. 1988. Immunogold labelling of bluetongue virus in cryosections from Culicoides variipennis (Coquillett) salivary gland. Proc. Electron Microscopy Soc. Amer., 372-373.
FRANCIS, B.R., BLANTON, W.E., LITTLEFIELD, J.L. and NUNAMAKER, R.A., Hydrocarbons of the cuticle and hemolymph of the adult honey bee (Apis mellifera Linnaeus). Annals Entom. Soc. Am., Accepted October 25, 1988.
SIEBURTH, P.J. and MARUNIAK, J.E. 1988. Growth characteristics of a cell line from the velvetbean caterpillar, Anticarsia gemmatalis Hubner (Lepidoptera: Noctuidae). In Vitro Cell Devel. Biol. 24:195-198.
SIEBURTH, P.J. and MARUNIAK, J.E. 1988. Susceptibility of a cell line of Anticarsia gemmatalis (Lepidoptera:Noctuidae) to three nuclear polyhedrosis viruses. J. Inverteb. Pathol. 52:453-458.
HOLBROOK, F.R. 1988. Bluetongue in the United States: Status, transmission and control through vector suppression. Bull. Soc. Vector Ecol. 13:350-353.
AKEY, D.H., LUEDKE, A.J. and OSBURN, B.I. 1988. Development of hypersensitivity in cattle to the biting midge (Diptera:Ceratopogonidae). Misc. Publ. Ent. Soc. Amer. 71:22-28.

30.071 CRIS0031030
ETIOLOGY AND PATHOGENESIS OF ANAPLASMOSIS

BEAR P D; TRUEBLOOD M S; SWIFT B L; Microbiology Biochemistry; University of Wyoming, Laramie, **WYOMING** 82070.
Proj. No.: WYO-020-069 Project Type: HATCH
Agency ID: CSRS Period: 17 DEC 82 to 30 SEP 86

Objectives: Cultivate A. marginale in vitro in cell culture. Determine the nutritional requirements of the organism. Cut A. marginale genome into different sized fragments and

construct map. Ligate different genomic fragments to cloning vectors. Transform ligated fragments into Ek2 host E. coli to obtain a library. Screen via antibody, for antigenic proteins produced by Ek2 host. Identify unique A. marginale proteins. Attempt to align proteins with genomic fragment map. Determine if acute anaplasmosis in yearling beef heifers interfers with estrous, ovulation and conception.

Approach: Infect nucleated cells, e.g. blood vessel endothelial cells, reticuloendothelial cells and macrophages. Quantitate growth by H Thymidine incorporation and staining. Observe changes in metabolic products and enzymes of infected and uninfected erythrocytes. Determine effects of these biochemicals on short term cultivars. Do complete and partial digests with Eco R(1), Bam Sal, Pst, etc. Construct fragment map. Ligate fragments to cloning vectors pBr 322, pBr 325 and charon phage. Transform ligated DNA into Ek2 host. Select via insertional inactivation and DNA packaging. Screen transformants using immunochemical methods. PAGE-SDS gels have revealed at least 4 A. marginale proteins to date. Each cloned A. marginale DNA fragment will be aligned with its encoded protein via modifications of the maxicell technique and gel electrophoresis.

Progress: 86/01 to 86/09. Anaplasmosis has a profound effect on reproduction in cattle. It can cause severe anemia often followed by abortion in pregnant beef cows, especially in the third trimester of gestation. It also causes testicular degeneration and loss of libido in mature beef bulls. The organism can only be grown in vitro for a short duration and exhibits a very narrow host range. Analysis of A. marginale protein revealed 7 unique protein bands on SDS PAGE immunoblots with estimated molecular weights of 106K,92K, 78K, 67K, 54K, 38K, and 34K. A. marginale DNA was found to contain 33% G + C and the genome was estimated to be 340 kb in size. A genomic library was constructed and one clone was found which produced the 92K protein but expression was poor. Limited vaccine trials with the cloned protein gave equivocal results. Labelled DNA from another clone was used as a genomic probe and could detect a 1% parasetmia in 10 mls of infected bovine blood. Metabolic studies of C14 labeled pyruvate showed that A. marginale does not synthesise fatty acids from pyruvate, de novo.

Publications: 86/01 to 86/09

TRUEBLOOD, M.S., SWIFT, B.L. and BEAR, P.D. 1972. Bovine Fetal Response to Anaplasma marginale. Am. J. Vet. Res. 23:618-625.

BEAR, P.D. 1973. Current concepts regarding the biochemical nature of A. marginale and their implications. In: Jones EW (ed) Proc Sixth Nat'l Anaplasmosis Conf. Oklahoma: Heritage Press pp 16-18.

BEAR, P.D. 1973. Observations of "tailed" Anaplasma marginale, In: Jones EW (ed) Proc Sixth Nat'l Anaplasmosis Conf. Oklahoma: Heritage Press pp 21-23.

CORBRIDGE, M.H., TRUEBLOOD, M.S. and BEAR, P.D. 1973. Characterization of Anaplasma marginale nucleic acid. In: Jones EW (ed) Proc Sixth Nat'l Anaplasmosis Conf. Oklahoma: Heritage Press pp 29-32.

KINGSTON, N., TRUEBLOOD, M.S. 1973. Attempts to transmit Anaplasma marginale. In: Jones, E.W. (ed) Proc Sixth Nat'l Anaplasmosis Conf. Oklahoma: Heritage Press, pp 42-45.

TRUEBLOOD, M.S. and BEAR, P.D. 1973. Cultivation of Anaplasmasis marginale. In: Jones E.W. (ed) Proc Sixth Nat'l Anaplasmosis Conf. Oklahoma: Heritage Press pp 54-58.

FOWLER, D. and SWIFT, B.L. 1975. Abortion in cows inoculated with Anaplasma marginale. Theriogenology. 4:59-67.

30.072 CRIS0138473

RECEPTORS FOR GROWTH HORMONE AND GONADOTROPINS

JI T H; Molecular Biology; University of Wyoming, Laramie, **WYOMING** 82070.

Proj. No.: WYO-247-89 Project Type: HATCH
Agency ID: CSRS Period: 30 JUN 89 to 29 MAY 94

Objectives: Determination of the complete genomic structure of the R(GH) gene and putative GH binding protein gene. The results may reveal the coding sequence of the putative GH binding protein. We would like to extend this study on the human genes to bovine genes. Investigation of the regulatory DNA sequences of these genes. Investigation of expression of these genes during fetal and neonatal development of the rat. Determination of the regulatory elements of hCG genes. Determination of interacting sites of hCG and the receptor.

Approach: DNA will be sequenced as we have routinely done in the past, employing double strands pGEM, sequenase II, alpha- S-ATP, deaza dGTP or dITP, and both the universal and reverse primers. We can routinely determine 350 bases in one direction in one tier system. It is possible to determine the sequence of a 1 kb insert without subcloning. Sequencing gels are read with a gel reader, the data entered into a DNA analysis program and aligned, and global sequence searches through GENBANK. We hope to determine not only the genomic sequences of the two genes but also the coding sequence of the putative GH binding protein.

31.001* CRIS0138386
EXPRESSION OF BOVINE HERPESVIRUS-4 GENOME IN TISSUE CULTURE

VAN SANTEN V; Pathobiology; Auburn University, Auburn, **ALABAMA** 36830.
Proj. No.: ALAV-0202 Project Type: STATE
Agency ID: CSVM Period: 01 JAN 89 to 30 SEP 91

Objectives: Establish Bovine Herpesvirus-4 (BHV-4) recombinant DNA clones and generate physical restriction endonuclease map. Characterize RNA transcribed from BHV-4 genome during latent, non-productive, and different stages of productive infection in tissue culture. Determine whether BHV-4 infection stimulates host cell DNA synthesis, growth transformation, or bovine leukemia virus (BLV) transcription.

Approach: Pst1 and Hind3 restriction enzyme fragments representing the entire genome of BHV-4 American prototype strain DN-599 will be cloned. RNA transcribed from the BHV-4 genome during different stages of infection in tissue culture will be characterized using the cloned restriction fragments by Northern blot analysis, S1 nuclease analysis, and hybridization of radiolabeled cDNA. Growth tranformation experiments will use primary bovine embryo cells, bovine lymphocytes, and mouse 3T3 cells, and intact BHV-4 virions as well as viral DNA fragments. Stimulation of BLV transcription will be tested by infecting cells containing a BLV-LTR-chloramphenicol acetyl transferase reporter gene plasmid.

31.002 CRIS0096432
CLONING AND EXPRESSION OF BOVINE CASEIN CDNA SEQUENCES

RICHARDSON T; Food Science & Technology; University of California, Davis, **CALIFORNIA** 95616.
Proj. No.: CA-D*-FST-4557-H Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 85 to 30 SEP 90

Objectives: To complete cloning of cDNA sequences coding for bovine alpha(s-1)-, beta- and kappa-caseins. To insert appropriate cDNAs into an expression vector for E. coli. To isolate genetically modified caseins for structure-function studies.

Approach: Conventional cloning procedures starting with casein mRNAs will be used. cDNAs will be inserted into pBR322 plasmid. Expression vector pCQV2 will be utilized for microbial production of caseins. Caseins will be modified using site directed mutagenesis for structure-function studies.

Progress: 88/01 to 88/12. Bovine (beta)-casein cDNA is expressed in yeast at a level of about 5 mg/liter using a vector containing a hexokinase regulatory sequence. A portion of the (beta)-casein is glycosylated by the yeast and binds concanavalin A. Upon treatment with trypsin both the glycosylated and normal (beta)-casein yields typical (gamma)-casein bands. From the known trypsinolysis of control (beta)-casein, the glycosylation evidently occurs in the N-terminal portion of the polypeptide. The 106-Phe mutant of (kappa)-casein is readily hydrolyzed by acid proteases to yield the same fragments as the 106-Met control. We are currently studying relative rates of hydrolysis of the mutant and control protein. Minigenes have been constructed utilizing the WAP regulatory sequences of the mouse WAP gene coupled to the bovine (alpha),(subscript s1)-casein cDNA. In addition, a mouse mammary tumor virus enhancer sequence has been inserted into the minigene construction to enhance possible expression. The hybrid minigenes will be introduced into mouse embryos and into mouse mammary secretory cells to study expression in transgenic mice and in the secretory cells.

Publications: 88/01 to 88/12

KANG, Y. C. and T. RICHARDSON. Jan. (1988). Molecular cloning and expression of bovine (kappa)-casein in Escherichia coli. Journal of Dairy Science 71(1):29-40.

CREAMER, L. K., R. JIMENEZ-FLORES and T. RICHARDSON. July (1988). Genetic modification of food proteins. Trends in Biotechnology 6(7):163-169.

JIMENEZ-FLORES, R. and T. RICHARDSON. (1988). Genetic engineering of milk proteins to improve behavior during processing. J. Dairy Sci. 71:2640-2654.

31.003* CRIS0134578
MOLECULAR CLONING OF THE GENOME OF THE ALCELAPHINE HERPESVIRUS I OF MALIGNANT CATARRHAL FEVER

ZEE Y; Microbiology & Immunology; University of California (vet-med), Davis, **CALIFORNIA** 95616.
Proj. No.: CALV-AH-104 Project Type: ANIMAL HEALTH
Agency ID: CSRS Period: 03 JUN 88 to 19 APR 93

Objectives: Restriction endonuclease mapping of field herpesviral isolates of malignant catarrhal fever (MCF) and comparison with prototype strains of MCF (e.g., WC11) to define differences and determine homology between viral strains. Molecular cloning of a representative herpesviral strain of MCF (WC11) to enable the development of a genetic probe which will recognize sequence homologies common for viral isolates of MCF.

Approach: The information obtained from this study will enable the development of a diagnostic probe which will recognize DNA sequences in tissue samples, leukocytes, or plasma from cattle or sheep suspected of harboring a latent infection with MCF or with clinical signs of an infection typical of MCF.

Progress: 88/01 to 88/12. A genomic probe specific for malignant catarrhal fever (MCF) virus was cloned by using purified viral DNA from MCF-virus strain WC11. Restriction endonuclease analysis of the purified viral DNA was used to identify the cloned viral genomic fragment. Dot blot hybridization by use of the

genomic probe (pRP-5) indicated that the probe hybridized specifically with WC11-MCF virus, as well as with one other isolate of MCF-associated herpesvirus. Hybridization also was observed to a non-MCF virus strain of bovine herpesvirus.

Publications: 88/01 to 88/12
SHIH, L.; IRVING, J.M.; ZEE, Y.C.; PRITCHETT, R.F. (1988). Cloning and characterization of a genomic probe for malignant catarrhal fever virus. American Veterinary Medical Association; Vol. 49; No. 10; Pages 1665-1668.

31.004* CRIS0093997
POXVIRUS AS A CLONING VECTOR FOR IMMUNIZATION AGAINST BOVINE HERPESVIRUS 1

BLAIR C D; PEARSON L D; College of Vet Medicine; Colorado State University, Fort Collins, **COLORADO** 80523.
Proj. No.: COLV2004 Project Type: SPECIAL GRANT
Agency ID: CSRS Period: 01 AUG 84 to 31 JUL 86

Objectives: Determine physical map location of glycoprotein genes on bovine herpesvirus 1 (BHV-1) DNA. Obtain molecular clones. Construct plasmids containing vaccinia virus promotor and non-essential DNA sequences. Insert clones BHV-1 DNA sequences into cloned vaccinia DNA sequences. Construct recombinant vaccinia virus mutants with incorporated BHV-1 genes. Determine immune response of laboratory animals and cattle to recombinant virus.

Approach: Molecular Clone BHV DNA fragments in expression plasmid. Select those whose products react with anti-glycoprotein monoclonal antibodies. Molecular clone vaccinia virus DNA fragments in pBR325. Construct recombinant plasmids by recombinant DNA methods. Simultaneously infect cultured cells with vaccinia virus and transfect with recombinant plasmids. Vaccinate mice with recombinant virus. Measure cell mediated immunity by Cr release.

Progress: 86/01 to 86/07. A library of monoclonal antibodies (MAb) was produced which denatured glycoproteins of bovine herpesvirus type 1 (BHV-1). Two MAb reacted with a 90,000/180,000 dalton protein complex and 10 MAb reacted with a 55,000/70,000/120,000 dalton protein complex. MAb which reacted with the 90K/180K complex partially competed with each other in protein binding assays, and neither neutralized virus infectivity. MAb which reacted with the 55K/70K/120K complex competed completely with each other in binding to proteins, but only 7 of them neutralized virus. The MAb have been used to map genes which code for glycoproteins as follows. Fragments of BHV-1 DNA with a size range of 500-1500 bp were produced by shearing. These fragments were inserted into the DNA of the expression vector lambda gt-11. The recombinant phage were screened by blotting plaques onto nitrocellulose and assaying for reactivity with MAb. Molecular hybridization showed that phage which produced a protein that was reactive with MAb to the 55K/70K/120K dalton complex contained a DNA insert from the Hind III A restriction fragment of BHV-1 DNA. More detailed mapping is in progress. A virus variant was isolated from BHV-1 stocks which infects mice. Further characterization of this variant is in progress.

Publications: 86/01 to 86/07
MCGRANE, V. 1985. Characterization of bovine herpesvirus 1 envelope glycoproteins with monoclonal antibodies. M.S. Thesis, Colorado State University, 114 p.
BLAIR, C.D., PEARSON, L.D., DUNN, D.C. and CHAPLIN, K.L. 1985. The mouse as a model for bovine herpesvirus-1 infections. Am. Soc. Virology Annual Meeting, Albuquerque, N.M., p. 41.
PEARSON, L.D., BLAIR, C.D., and CHAPLIN, K.L. 1985. Infection of BALB/C mice with bovine herpesvirus 1. Rocky Mountain Immunologists Abstracts, Aspen, CO, p. 10.
BLAIR, C.D., MCGRANE, V., TERAMOTO, Y. and MUIRHEAD, J. 1986. Monoclonal antibodies to denatured bovine herpesvirus-1 glycoproteins. Am. Soc. Virology Annual Meeting, Santa Barbara, CA, p. 23.

31.005* CRIS0136061
RANGE OF HYPOBIOTIC STRAINS OF OSTERTAGIA OSTERTAGI ASSESSED USING DNA MARKERS

DAME J B; COURTNEY C H; Horticulture & Landscape Architecture; University of Florida, Gainesville, **FLORIDA** 32611.
Proj. No.: FLA-VME-02768
Project Type: SPECIAL GRANT
Agency ID: CSRS Period: 01 SEP 88 to 31 AUG 91

Objectives: The primary objectives of this project are to: (1) identify DNA markers which are capable of distinguishing strains of Ostertagia ostertagi with the capacity to undergo hypobiosis (arrested larval development) during the summer months from strains which arrest their development during winter, and (2) use these markers to determine the prevalence and geographic range of these strains in an area where their distribution overlaps (Virginia).

Approach: Clone multi-copy genes and repetitive DNA sequences for probes to differentiate Ostertagia ostertagi strains by DNA hybridization analysis. Use DNA probes to analyze samples of helminth eggs obtained from cattle from known farms of origin in Virginia.

31.006* CRIS0136409
SEX-SPECIFIC DNA IN LIVESTOCK ANIMALS

MCGRAW R A; College of Vet Medicine; University of Georgia, Athens, **GEORGIA** 30602.
Proj. No.: GEOV-0186 Project Type: STATE
Agency ID: CSVM Period: 01 JUL 87 to 30 JUN 91

Objectives: This project is aimed at identifying and characterizing sex-specific DNAs in economically important livestock

species. The basic genetic information can be used to develop sex-specific DNA probes with potential application in assays for sex-fractionation of semen and/or sex determination of embryos.

Approach: The approach is to compare DNAs derived from male and female animals of each species by a variety of molecular genetic methods, including restriction analysis, cloning, sequencing, and hybridization techniques. DNA sequences unique to one of the sexes are then characterized and developed as sex-specific hybridization probes.

Progress: 87/07 to 88/12. This research is aimed at identifying and characterizing sex-specific DNAs in economically important livestock species. The genetic information is used to develop sex-specific DNA probes with potential application in assays for sex-fractionation of semen and in sex-identification of embryos. Methods include a variety of DNA manipulations: restriction enzyme digestions, electrophoretic separations, construction and propagation of recombinant DNA in bacteria, DNA sequece analysis, chemical DNA synthesis, enzymatic DNA amplification, and hybridizations using radioactively labelled probes. At this time, we have developed sex-specific probes in pigs and chickens. The procine probe has been used successfully for sex-identification of procine embryos and efforts are underway to attempt sex-fractionation of boar semen. Preliminary data suggests that we will be able to develop similar probes in horses and cattle.

Publications: 87/07 to 88/12

MCGRAW, R.A., JACOBSON, R.J. and AKAMATSU, M. 1988. A male-specific repeated DNA sequence in the domestic pig. Nucleic Acids Research 16(21):10389.

31.007 CRIS0093986
MOLECULAR AND CELLULAR BIOLOGY OF THE NONLACTATING MAMMARY GLAND

HURLEY W L; Animal Science; 1301 West Gregory Drive, Urbana, **ILLINOIS** 61801.
Proj. No.: ILLU-35-0370 Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 87 to 30 SEP 92

Objectives: To define and characterize molecular and cellular mechanisms of mammary function during the nonlactating period.

Approach: Proteins in mammary tissue during the nonlactating period will be isolated and characterized. Mammary tissue will be characterized immunohistologically and ultrastructurally. Molecular probes (monoclonal antibodies and DNA probes) will be developed for proteins involved in mammary function during the nonlactating period.

Progress: 87/10 to 88/09. The objectives are to identify, characterize and develop probes for proteins secreted by the nonlactating bovine mammary gland and to identify mechanisms of secretion of those proteins. Lactoferrin was isolated from bovine mammary secretions by heparin-agarose affinity chromatography. Two molecular weight forms of lactoferrin (83 and 87 kilodaltons) were identified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Endoglycosidase digestion and concanavalin A-binding indicated that both molecular weight forms of the lactoferrin were glycosylated, but the difference between the two forms may not be exclusively due to oligosaccharide composition. Bovine mammary explant cultures incubated with radiolabled methionine was used in a preliminary study of lactoferrin secretion. Lactoferrin was immunoprecipitated from homogenates of labeled tissue, proteins were separated by SDS-PAGE and labeled proteins were visualized by fluorography. Lactoferrin synthesis increased during involution until at least day 14 and then declined. Bovine mammary tissue was collected and prepared for electron microscopy. Ultrathin sections were immunolabeled using rabbit antisera specific for the bovine milk proteins, casein (CN), (Beta)-lactoglobulin ((Beta)-LG) and (Alpha)-lactalbumin ((Alpha)-LA). Primary antibodies were visualized with Protein-A conjugated to 5 nm colloidal gold.

Publications: 87/10 to 88/09

HURLEY, W. L. and L. A. SCHULER. (1987). Molecular cloning and nucleotide sequence of a bovine alpha-lactalbumin cDNA. Gene 61:119-122.
HURLEY, W. L. and R. M. DOANE. (1988). Recent developments in the role of vitamins and minerals in reproduction. J. Dairy Sci., in press.

31.008 CRIS0097985
MAMMARY GLAND IMMUNOGLOBULIN TRANSPORT: MOLECULAR BIOLOGY OF THE IGG RECEPTOR

HURLEY W L; Animal Science; 1301 West Gregory Drive, Urbana, **ILLINOIS** 61801.
Proj. No.: ILLU-35-0981
Project Type: ANIMAL HEALTH
Agency ID: CSRS Period: 01 MAR 86 to 30 SEP 88

Objectives: To characterize the bovine mammary immunoglobulin receptor, clone cDNA probes for the receptor, and identify the gene for the receptor.

Approach: Receptor will be partially purified form prepartum mammary tissue and characterized by gel electrophoresis. A cDNA library will be screened using a differential hybridization scheme. Identified cDNAs will be used to screen a bovine genomic library. Cloning and characterization of DNAs will be by established techniques.

Progress: 88/01 to 88/12. The objectives are to isolate, characterize and purify the bovine mammary IgG receptor. Experiments to isolate the receptor have used zwitterionic and nonionic detergents to solubilize membrane proteins from crude mammary membrane preparations. Solubilized proteins were chromatographed on an anti-bovine IgG-Fab immuno-affinity column. Specifically bound proteins were eluted and chromatographed on a

bovine IgG1-Fc affinity column and eluted proteins were characterized by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and silver staining. Little protein was retained on an IgG-Fab affinity column. The presence of IgG1 in solubilized mammary membrane preparations was confirmed by co-migration (on SDS-PAGE) of purified IgG1 heavy and light chains with two proteins eluted from the Fc column and by elution of immunoglobulins from the anti-bovine IgG-Fab column. Secretory component, IgA and IgM also were present in solubilized mammary membrane preparations. Several proteins that were retained by the Fc affinity column were glycosylated, as determined by lectin-affinity protein blotting. Proteins bound to the Fc column included a 54 kilodalton protein. This protein was not found in fractions binding to the Fab affinity column. The 54 kilodalton protein is believed to be an IgG Fc receptor. A separate study utilized immunoperoxidase histochemistry to localize IgG1 in mammary tissue during involution. In lactating tissue, IgG1 was localized primarily in the stromal areas.

Publications: 88/01 to 88/12
No publications reported this period.

31.009* CRIS0137128
MOLECULAR CLONING AND SEQUENCING OF BOLA CLASS II GENES

LEWIN H A; Animal Science; 1301 West Gregory Drive, Urbana, **ILLINOIS** 61801.
Proj. No.: ILLU-35-0347 Project Type: HATCH
Agency ID: CSRS Period: 01 JAN 89 to 30 SEP 92

Objectives: The objectives of the proposed research are to: 1) construct a cDNA library from the BL-3 cell line (a cell line which expresses high levels of BoLA class II molecules), 2) identify and subclone all unique class II cDNAs (1.3-1.5 kb) in this cDNA library, 3) sequence unique clones (products of different loci and alleles) and compare sequences with other MEC class II sequences in the GenBank database, and 4) develop locus-specific and allele-specific probes for DNA-typing and mapping the class II region of cattle.

Approach: The cDNA library, cloned in lambda gt 10, will be screened by colony hybridization using human class II cDNA probes. Unique and allelic clones will be subcloned in pUC19 and sequenced in their entirety by the dideoxy chain terminating method. Sequence analysis will be performed using the DNASTAR computer software.

31.010 CRIS0088115
ISOLATION AND CHARACTERIZATION OF THE BOVINE PLACENTAL LACTOGEN GENE

HURLEY W L; Dairy Science; 1301 West Gregory Drive, Urbana, **ILLINOIS** 61801.
Proj. No.: ILLU-35-0364 Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 82 to 30 SEP 86

Objectives: The objective is to develop, characterize and identify a copy DNA (cDNA) of bovine placental lactogen (PL). And, to use this cDNA to quantitate bovine PL mRNA levels in placentae at different gestation stages.

Approach: A cDNA library will be developed from bovine placental poly A RNA by standard procedures, and bPL cDNAs identified by hybridization to genomic probes already developed. Full-length cDNA will be mapped with restriction enzymes, and nucleotide sequence determined. This cDNA will be used to purify bPL mRNA, and the cell-free translation product will be identified by immunoprecipitation. Bovine placental RNA from different gestation stages will be quantitated for bPL mRNA by hybridization to radiolabeled bPL cDNA.

Progress: 82/10 to 86/09. The objectives were to clone and characterize a cDNA for bovine placental lactogen (PL), and to identify and characterize the bovine PL gene. To identify the cDNA, a cDNA similar to either bovine prolactin or growth hormone was screened for in a fetal placental cDNA library using low stringency hybridization. A clone similar, but distinct from bovine prolactin was found. Further study yielded several additional distinct cDNA's more similar to prolactin than growth hormone, but differing as much from one another as from prolactin. This event for a family of genes is supported by isolation of several distinct genomic clones from a bovine genomic library using the placental cDNA's, and appearance of a very complex pattern on a genomic Southern blot probed with one of our placental cDNAs. This study of these genes in the bovine is the only such study in a nonprimate, nonrodent species. Like rodents, but unlike primates, the bovine placental hormones are more closely related to prolactin rather than growth hormone. The finding of multiple related genes contrasts with the isolation of a single protein hormone in this species. The functions of these placental hormones are not known in any species. However, these hormones have been postulated to play roles during pregnancy analogous to those of metabolism, fetal growth, and development of the mammary gland in preparation for lactation.

Publications: 82/10 to 86/09
GRUMMER, R.R., HURLEY, W.L., DAVIS, C.L. and MEACHAM, C.A. 1986. Effect of isolation temperature of the determination of bovine plasma very low density lipoprotein concentrations. J. Dairy ci. 69:2083-2090.
GRUMMER, R.R., MEACHAM, C.A., HURLEY, W.L. and DAVIS, C.L. 1986. Electrophoretic characterization of proteins from non-lactating and lactating bovine lipoproteins isolated by gel filtration chromatography. J. Dairy Sci. 69 (Suppl. 1):169.

SCHULER, L.A. and HURLEY, W.L. 1986. Bovine fetal placenta produces at least two discrete prolactin-related hormones. 68th Annual Meeting, Endocrine Society, Anaheim, CA. Abs. 435.

31.011 CRIS0096153
RELATIONSHIPS OF ENERGY-YIELDING NUTRIENTS TO PERFORMANCE OF LACTATING COWS

YOUNG J W; BEITZ D C; BEITZ D C; Animal Science; Iowa State University, Ames, **IOWA** 50011.
Proj. No.: IOW02736 Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 85 to 30 SEP 90

Objectives: To determine the role(s) of: a. glucose in milk production of high-producing cows; b. the liver in establishing productive performance of cows; c. availability of energy-yielding substrates on incidence of ketosis; d. in vivo kinetic techniques to evaluate energy metabolism of cows.

Approach: a. Glucose shortages will be created by injecting phlorizin into cows fed either low or high energy diets. b. Cows with small, intermediate, or large increases in liver lipid or decreases in liver glycogen will be used to measure milk production and changes in key blood constitutents. c. Use isocaloric amounts of high and low energy diets to evaluate susceptibility to ketosis. d. Confirm that multicompartmental in vivo kinetic analyses accurately reflect changes in metabolism of cows. Quantify changes in energy metabolism caused by "nutrient partitioning" agents.

Progress: 88/01 to 88/12. Four Holstein cows, fed to meet DE requirements, were used to quantify changes that occur in the metabolism of propionate, glucose, and CO(subscript 2) when glucose was infused into the peripheral blood supply. Neither production nor composition of milk was changed by glucose. Irreversible losses of rumen propionate and blood CO(subscript 2) were not changed. Glucose treatments increased glucose irreversible loss over controls in proportion to the amount of glucose infused, but did not change endogenous glucose production. Such results provide insights into how nutrients are partitioned in high-producing cows, Research efforts on cell mitochondria have focused on the relationship of mitochondrial DNA (mtDNA) to milk production in cows. We have developed a novel and convenient method for the isolation of mtDNA from white blood cells. Then we isolated, cloned, and determined the nucleotide sequence of 1000 base pairs from each of 30 distinct maternal lineages of Holstein cows. Of 29 point mutations found so far, one (HpaII) correlates significantly with milk fat percentage and has potential usefulness as a genetic selection aid for dairy cattle. Restriction fragment length polymorphism (RFLP) analysis of cloned mtDNA has revealed several polymorphisms potentially useful for tracing or confirming maternal origins of cattle. Efforts to determine mechanisms of mtDNA effects on milk production are underway.

Publications: 88/01 to 88/12

AMARAL, D. M. (1988). Metabolic effects associated with changes in the availability of glucose for lactating dairy cows. Ph.D. Dissertation. Iowa State University, Ames. 141p.

COOLEY, M. H., AMARAL, D. M., and YOUNG, J.W. (1988). Effects of intraduodenal and intravenous infusions of glucose on irreversible loss and endogenous production of glucose in mid-lactation dairy cows. J. Dairy-Sci. 71(Suppl. 1):246.

AMARAL, D. M., COOLEY, M. H., TRENKLE, A. H., MCGILLIARD, A. D., and YOUNG, J. W. (1988). Comparisons of short-term and long-term adjustments to intravenous infusions of glucose in high-producing cows. J. Dairy Sci. 71(Suppl. 1):247.

KOEHLER, C. M., LINDBERG, G. L., MAYFIELD, J. E., MYERS, A. M., FREEMAN, A. E., and BEITZ, D. C. (1988). Evaluation of a rapid method for isolation of mitochondrial DNA. FASEB J. 2:A1126 (Abstract).

LINDBERG, G. L., SHANK, B. B., ROTHSCHILD, M. F., MAYFIELD, J. E., FREEMAN, A. E., KOEHLER, C. M., and BEITZ, D. C. (1988). Characteristics of mammary mitochondria in lines of mice genetically divergent for milk production. J. Dairy Sci.

BROWN, D. R., KOEHLER, C. M., LINDBERG, G. L., FREEMAN, A. E., MAYFIELD, J. E., MYERS, A. M., SCHUTZ, M. M., and BEITZ, D. C. (1988). Molecular analysis of cytoplasmic genetic variation in Holstein cows. J. Anim. Sci. (In Press).

31.012* CRIS0131898
GENETIC IMPROVEMENT OF PROPIONIBACTERIA AND OTHER MICROORGANISMS IMPORTANT IN FOODS

GLATZ B A; Food Technology; Iowa State University, Ames, **IOWA** 50011.
Proj. No.: IOW02827 Project Type: HATCH
Agency ID: CSRS Period: 01 JUL 87 to 30 JUN 92

Objectives: To improve strains of Propionibacterium important in the dairy industry and in propionic acid production. To understand the genetic organization of this and of other Gram-positive organisms. To develop means of gene transfer among these organisms and other Gram-positive organisms. To identify and characterize important genetic determinants in these organisms.

Approach: Plasmids native to propionibacteria will be isolated and characterized. Genes carried on plasmids will be sought, and characterized when found. Conjugations using conjugative plasmids from other Gram-positive organisms or from the propionibacteria will be established. Transformation of DNA into protoplasts or whole cells will be performed. Bacteriophage specific for propionibacteria will be sought. Mutants altered in important traits will be constructed.

Progress: 88/01 to 88/12. The goal of the research is to investigate the genetics of the propionibacteria, which are important industrial organisms. Screening of the culture collection of 119 strains of propionibacteria

for the presence of plasmid DNA has been completed. Twenty strains were found to contain plasmids, and at least 10 unique plasmids were identified in these strains. Seven plasmids were partially characterized by restriction endonuclease analysis, and restriction maps were constructed for four of these. Hybridization studies were conducted to determine the relationships among the seven plasmids that were partially characterized. Five of these plasmids were cured from their respective strains by chemical treatment, and all plasmidcarrying strains and cured derivatives were checked for antibiotic resistances, carbohydrate fermentations, and bacteriocine production. Three plasmid-associated traits have been identified: lactose fermentation and a possible cell-surface component on one plasmid, and cell clumping on another plasmid. The culture collection has been screened for the presence of temperate and/or inducible bacteriophage and bacteriocin production, and samples of rumen fluid, Swiss cheese whey, silage, and lake water have been tested for the presence of lytic phage or other inhibitory components. One strain, recently isolated from Swiss cheese, appears to contain a defective bacteriophage.

Publications: 88/01 to 88/12

GLATZ, B.A. and ANDERSON, K.I. (1988). Isolation and characterization of mutants of Propionibacterium strains. J. Dairy Sci. 71: 1769-1776.

31.013 CRIS0089692

STUDY OF THE GENETICS AND GENETIC MODIFICATION OF MICROORGANISMS IMPORTANT IN FOODS

GLATZ B A; HAMMOND E G; PATTEE P A; Food Technology; Iowa State University, Ames, **IOWA** 50011.

Proj. No.: IOW02611 Project Type: STATE
Agency ID: SAES Period: 01 JUL 83 to 30 JUN 87

Objectives: To locate the genes involved in enterotoxin production by Staphylococcus aureus. To determine if plasmids are involved in enterotoxin production in S. aureus. To develop means of controlling enterotoxin production in S. aureus. To devise methods for mutagenizing Propionibacterium strains and other dairy starter cultures. To produce desirable mutants of Propionibacterium. To examine Propionibacterium strains for the presence of plasmids.

Approach: Genetic analysis will be performed by means of conventional mapping procedures such as transformation and transduction as well as by protoplast fusion. Analysis for the presence of plasmids will be by agarose gel electrophoresis of isolated DNA. Mutagenesis will be done by exposure to chemical mutagens and to ultraviolet light.

Progress: 87/07 to 87/12. No progress reported this period.

Publications: 87/07 to 87/12

VORHEES, K.I. and GLATZ, B.A. 1987. Generation, isolation and characterization of auxotrophic mutants of Propionibacterium. J. Dairy Sci. 70 Suppl. 1:79.

PAI, S.L. and GLATZ, B.A. 1987. Production, regeneration and transformation of protoplasts of Propionibacterium strains. J. Dairy Sci. 70 Suppl. 1:80.

REHBERGER, T.G. and GLATZ, B.A. 1987. Characterization of plasmid DNA in Propionibacterium freudenieichii subsp. globosum P93: evidence for plasmid-linked lactose utilization. J. Dairy Sci. 70 Suppl. 1:80.

GLATZ, B.A. 1987. Studies on the genetics of Propionibacteria for their improvement as industrial microorganisms. J. Dairy Sci. 70 Suppl. 1:96.

31.014* CRIS0048466

PRODUCTION OF BRUCELLA SURFACE PROTEINS WITH RECOMBINANT DNA TECHNOLOGY

TABATABAI L B; MAYFIELD J E; Humanities & Science; Iowa State University, Ames, **IOWA** 50011.

Proj. No.: 3630-34000-003-01S
Project Type: COOPERATIVE AGREE.
Agency ID: ARS Period: 22 FEB 84 to 30 SEP 88

Objectives: Establish a clone library for the Brucella abortus genome and isolate clones that code for surface proteins. Subclone useful genes into E. coli plasmids to produce Brucella proteins and explore methods for purifying these proteins..

Approach: DNA extracted from B. abortus will be cleaved with restriction endonucleases, randomly cloned into lambda phage, and propagated on E. coli K12. The colone library will be screened for specific Brucella surface proteins. Brucella proteins expressed will be identified and characterized in comparison with similar products of direct extraction. E. coli plasmid-mediated synthesis of Brucella proteins will be evaluated and purification methods will be developed..

Progress: 88/01 to 88/12. Two new lambda clones have been identified which express 70kDa and 66kDa Brucella proteins, respectively. The antiserum (R213) used to identify these clones were raised against a small group of 60-80kDa proteins which are identified by cattle antisera from vaccinated and infected animals. A large amount of effort was also expended unsuc- cessfully in attempting to clone the gene coding for BCSP34. It seems likely that this protein is not expressed in the Brucella DNA library we are using. We completed a study to determine the cellular location of BCSP20, BCSP31, and BCSP45 in recombinant E. coli cells. All three proteins are located in the periplasm, and all three cause E. coli to nonspecifically leak periplasmic proteins into the growth medium. We have also constructed some of the subclones necessary to sequence BCSP20 and BCSP45. We have introduced plasmids coding for all three proteins into several strains of S. typhimurium. High expression of

BCSP45 seems to be lethal, while BCSP20 and BCSP31 express as well as in E. coli. Preliminary data indicates that mice infected with S. typhimurium expressing BCSP31 produce circulating antibodies against this protein.

Publications: 88/01 to 88/12

MAYFIELD, J.E., BRICKER, B.J., GODFREY, H., CROSBY, R.M., KNIGHT, D.J., HALLING, S.M., BALINSKY, D. and TABATABAI, L.B. 1988. Cloning, expression, and nucleotide sequence of a gene cloning for an immunogenic Brucella protein. Gene 63:1-9.

31.015* CRIS0004941

CONSULTATION AND RESEARCH IN MATHEMATICAL AND STATISTICAL GENETICS

POLLAK E; Statistics; Iowa State University, Ames, **IOWA** 50011.
Proj. No.: IOW01448 Project Type: STATE
Agency ID: SAES Period: 01 JUL 59 to 01 JAN 99

Objectives: Study of population genetics, with particular reference to balanced polymorphisms maintained by natural selection occurring in human and other species. Consultation on mathematical problems arising from workers in genetics.

Approach: Procedure will consist partly of the examination of theoretical models and will be partly in cooperation with individuals who have collected or are collecting data on genetic populations.

Progress: 88/01 to 88/12. E. Pollak provided assistance to Dr. A. R. Hallauer of the Department of Agronomy, who asked a question concerning covariances between relatives when a population originally has a Hardy-Weinberg structure and successive generations are produced by self fertilization. Let FS(subscript n) be the mean of a full sib family resulting from a cross between two plants that are produced after n generations of selfing. It was verified that the covariance between FS(subscript 0) and FS(subscript 4) is equal to the covariance between full sib offspring of individuals of generation 0. Assistance was also provided to Mr. Brad Hedges, a student in the Department of Agronomy. He was faced with the problem of calculating what family size is large enough so that, if there are two possible sets of underlying frequencies of K types of offspring of a cross, the probabilities of the two kinds of misclassification are each 0.025. Professor C. P. Cox of the Department of Statistics and E. Pollak collaborated in solving this problem. Previously, the solution was known only if there are two types of offspring.

Publications: 88/01 to 88/12

JUNG, Y. C., ROTHSCHILD, M. F., FLANAGAN, M. P., POLLAK, E. and WARNER, C. M. Genetic variability between two breeds based on restriction fragment length polymorphisms (RFLPs) of major histocompatability complex class I genes in the pig.

31.016* CRIS0060069

BOVINE RESPIRATORY DISEASE, RISK FACTORS, PATHOGENS, DIAGNOSIS AND MANAGEMENT

ROSENBUSCH R F; ROTH J A; PAUL P S; Veterinary Medical Research Institute; Iowa State University, Ames, **IOWA** 50011.
Proj. No.: IOW01905 Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 86 to 30 SEP 91

Objectives: Antigens, immunogens, virulence factors and corresponding genomic sequences of the etiologic agents of bovine respiratory disease (BRD) will be identified. Specific and nonspecific resistance mechanisms which aid in control and prevention of bovine respiratory disease will be defined and enhanced. The basic mechanisms of respiratory injury in BRD will be identified and related to the evolution of the pathophysiologic basis of the lesions. Techniques and practices related to diagnosis, prevention, treatment and management of BRD will be evaluated.

Approach: Specific antigens of Nycoplasma dispar and Hemophilus somnus will be characterized using monoclonal antibodies and physiochemical separation techniques. Genome fragments of M. dispar will be cloned to prepare expression libraries and hybridization probes to determine prevalence of M. dispar variants. Subpopulations of lymphocytes will be obtained from BVD-infected calves and examined for functional responsiveness to BRD agents. Characteristics of recombinant lymphokines will be studied. Immunomodulators will be studied in cattle vaccinated or infected with BRD agents or their antigens, and immunosuppression by BVD and BRSV will be assessed. Rapid detection of BVD will be attempted with monoclonal antibodies and DNA probes.

Progress: 88/01 to 88/12. Cytopathic, Oregon C24V, and noncytopathic New York 1 (NY1), strains of bovine virus diarrhea virus (BVDV) were studied in vitro, using bovine turbinate cells (BTU) and bovine endothelial cells (BEC). Virus behavior in the two cell types was compared using one-step virus growth curves, radioimmunoprecipitation (RIP), and cytotoxicity assays. Both C24V and NY1 replicated to overall higher titers in BEC's than in BTU's, but production of new virions appeared to occur in both cell types within the same time frame. In BEC's the NY1 virus synthesizes a polypeptide with an approximate molecular mass of 89-92 kD. This polypeptide is absent in NY1 infected BTU's. It has been shown previously (and confirmed in this work) that a 80-87 kD is synthesized by cytopathic BVD strains, while noncytopathic strains fail to produce this protein. In addition, transient cytoplasmic vacuolation could be observed in NY1 infected BEC's, but not in BTU's. In contrast, no cytotoxic activity induced by NY1 infection could be detected in either cell type using a 51-Chromium uptake assay. It was concluded that based on RIP data and morphological evaluations, viral polypeptide expression and cytopathology can be dependent on the host cell type.

Publications: 88/01 to 88/12
No publications reported this period.

31.017* CRIS0133008
JOHNE'S DISEASE: PRODUCTION OF ANTIGENS FOR USE IN DIAGNOSTIC TESTS

THOEN C O; Veterinary Microbiology & Preventive Medicine; Iowa State University, Ames, **IOWA** 50011.
Proj. No.: IOWV-416-23-07 Project Type: SPECIAL GRANT
Agency ID: CSRS Period: 01 JUL 87 to 30 JUN 91

Objectives: To develop a more specific diagnostic test without the undesirable cross reactions which interfere with the interpretation of diagnostic tests for paratuberculosis and to isolate these genes in E. coli. To prepare polyclonal antibodies against the same strain of M. paratuberculosis for screening the genomic library. To evaluate the proteins produced by selected genes in E. coli in a ELISA to detect specific antibodies in sera of M. paratuberculosis infected cattle.

Approach: DNA fragments of M. paratuberculosis will be cloned into plasmid pUC19. A genomic library will be prepared in E. coli using this plasmid. Mycobacterial proteins produced in E. coli will be evaluated by immunoblot and by ELISA using polyclonal antibodies obtained from cattle exposed to M. paratuberculosis.

Progress: 88/01 to 88/12. Genomic DNA was prepared from spheroplasts of M. paratuberculosis. The purified chromosomal DNA was partially digested with EcoRI and ligated into the EcoRI site of plasmid pUC19. The recombinant molecules were used to transform competent E. coli cells. The resulting transformants containing mycobacterial DNA inserts were screened by immunoblotting using serum from a bovine in which paratuberculosis was diagnosed. The molecular weights of immunogenic proteins were determined by analysis in a western blot. The sizes and physical maps of DNA segments encoding such proteins were compared.

Publications: 88/01 to 88/12
PIERCE, J.K., ANDREWS, R.E., THOEN, C.O. and WAITE, K. Cloning and Expression of Mycobacterium paratuberculosis Genes in Escherichia coli. Abstracts of 88th Annual Mtg. American Society for Microbiology, p. 132.

31.018* CRIS0133790
USE OF A HUMAN POLYMORPHIC DNA MARKER IN THE BOVINE SPECIES

TROYER D L; SMITH J E; LEIPOLD H W; Anatomy & Physiology; Kansas State University, Manhattan, **KANSAS** 66506.
Proj. No.: KAN081836Project Type: ANIMAL HEALTH
Agency ID: CSRS Period: 01 DEC 87 to 30 SEP 88

Objectives: To develop a synthetic oligonucleotide probe based on the core sequence of a tandemly repetitive region near the human gene for DNA fingerprinting and segregation analysis in the bovine genome.

Approach: Bovine genomic DNA will be extracted, purified, size fractionated on agarose gels, and transferred to nylon. A synthetic probe based on the insulin polymorphism will be labeled by the random primer technique and southern blot analysis will be utilized to analyze the bovine genome for similar polymorphisms.

Progress: 87/12 to 88/09. The human VNTR (variable number tandem repeat) probes PEKMDA, pJCZ 3.1, and PYNH24 were used in Southern blot analysis of bovine DNA to ascertain their usefulness as polymorphic DNA markers in that species. The PJCZ31 probe, based on a region near the human zetaglobin psuedogene revealed a polymorphic pattern in unrelated bovine DNA digestd with the enzyme Ms PI. The other two probes, based on region in the hepatitis B virus genome revealed moderate polymporhisms within the bovine genome when the same restriction endonuclease was used. Although it was difficult to obtain excellent signal to noise ratios with these heterologous probes, they should be useful in gene mapping efforts in this species. In addition, the human marker PCMM was analyzed in the equine species and found to cross-hybridize with DNA from this species.

Publications: 87/12 to 88/09
TROYER, D., HOWARD, D, LEIPOLD, H.W. and SMITH, J.E. A human minisatellate sequence reveals DNA polymorphism in the equine species. Zentrabl Vet. Med. (in press).

31.019* CRIS0137865
A SEARCH FOR RESTRICTIVE FRAGMENT LENGTH POLYMORPHISMS (RFLP) IN THE BOVINE GENOME

SMITH J E; LEIPOLD H W; TROYER D; Pathology; Kansas State University, Manhattan, **KANSAS** 66506.
Proj. No.: KAN00794 Project Type: HATCH
Agency ID: CSRS Period: 01 JUL 89 to 30 SEP 92

Objectives: To locate RFLP's in the bovine genome for genetic markers of genetic diseases in cattle.

Approach: High molecular genomic DNA will be extracted from bovine leukocytes, digested with five-fold excess of various restriction endonucleases, and separated by agarose gel electrophoresis. The separated fragmented DNA will be transferred to nitrocellulose for Southern analysis using probes that reveal DNA polymorphisms in man.

31.020 CRIS0134414
GLYCOPROTEINS SPECIFIED BY BOVINE HERPES VIRUS 1 (BHV-1)

KOUSOULAS K G; Veterinary Microbiology & Parasitology; Louisiana State University, Baton Rouge, **LOUISIANA** 70803.
Proj. No.: LAV-0961-SVM
Project Type: ANIMAL HEALTH
Agency ID: CSRS Period: 01 MAR 88 to 30 JUN 91

Objectives: Clone cDNA's encoding all BHV-1 glycoproteins and express them via SV-40 based eucaryotic expression vectors.

Approach: The central novel approach that will be employed is transient expression of cDNA's derived from polyA+ RNA isolated from BHV-1 infected cells in Cos cells and physical selection of expressing cells by adhesion to antibody-coated dishes (immunological panning). The cDNA's are recovered from the cells that remain adhered to antibody coated dishes (Hirt extraction). This approach has allowed a large number of surface antigens cDNA's to be cloned in a short period of time (7,8). A major convenience of this method is the recovery of the cDNA of interest in a form containing the necessary sequences for surface expression.

Progress: 87/07 to 88/06. The genome of Bovine Herpes Virus 1 (BHV-1) was cloned as Hind III fragments into prokaryotic vectors PUC18 and PUC19. DNA cloned fragments containing the coding sequences for glycoproteins gI, gIII and gIV were identified and characterized by restriction enzyme mapping. Glycoprotein gI way cloned into eukaryotic expression vector p91023 and transfected into COS cells under transient conditions. Preliminary indirect immunofluorescence results indicate that this clone expressed the gI glycoprotein.

Publications: 87/07 to 88/06
NO PUBLICATIONS REPORTED THIS PERIOD.

31.021 CRIS0131677
REPETITIVE SEQUENCES IN THE BOVINE GENOME

BLAKE R; Biochemistry; University of Maine, Orono, **MAINE** 04469.
Proj. No.: ME08405 Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 87 to 30 SEP 92

Objectives: Specific objectives include gaining evidence that the long 1.4 kb repetitive sequence in the bovine genome is composed of shorter repeats of greater evolutionary age; the determination of maximum and minimum sizes of repeats; measurements of sequence divergence in the 1.4 kb sequence; and search for homologous sequences among the data base of bovine structural gene sequences.

Approach: Specific methods of approach include conducting high resolution preparative density gradient fractionation of bovine genomic DNA; restriction mapping of repetitive sequences; cloning of repetitive sequences; high resolution melting studies for sequence divergence; and computer search of the Los Alamos GenBank data base tape for homologous sequences.

Progress: 87/10 to 88/10. Bovine Satellites I (1400 pb repeat), II (46 bp repeat) and III (2350 bp repeat), have been purified to >90% by bisbenzamide dye CsC1 density gradient certrifugation. The (G+C) content and distributions of all three have been determined. The sequence of satellites I and II from bovine and bison, determined by the Maxim-Gilbert chemical method, are being compared for the level of sequence divergence in these two species. Sequence divergence of these two satellites have also been determined by the indirect heteroduplex melting assay. There is almost quantitative agreement between the latter method and sequence comparisons, supporting the efficacy of the latter method. The amounts of these three satellites in the bovine genome have been determined and are being determined in the bison genome at this time. The amounts of all three satellites are significantly less in bison than bovine, and intermediate in beeffalo, a cross between the two. Sequence divergence among the population of repeats is low in all three cases, being in the range of 1-2.5%. Divergence in bison is slightly greater than in bovine. From these and other results we have concluded that bison is the more primitive species; closer to a common ancester for the two.

Publications: 87/10 to 88/10
BLAKE, R.D. and DELCOURT, S.G. (1987). Biopolymers 26, 2009-2026, Loop Energy in DNA.
BLAKE, R.D. and DELCOURT, S.G. (1988) Biochemical Pharmacology 37(9), 1843. Elasticity of DNA in Nonhelical Loops.

31.022 CRIS0088114
PHYSICAL, CHEMICAL, EVOLUTIONARY AND STATISTICAL STUDIES OF THE BOVINE GENOME

BLAKE R D; Biochemistry; University of Maine, Orono, **MAINE** 04469.
Proj. No.: ME08405 Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 82 to 30 SEP 87

Objectives: The proposed work is designed to increase our knowledge of aspects of structure and dynamic behavior of the bovine genome. We will endeavor to determine the precise number of repetitive sequence groups, their length, base composition and sequence, the sequence divergence throughout each repetive sequence group, their evolutionary origins and ages, and the fractional amounts of each group in total bovine genomic DNA.

Approach: Total bovine genomic DNA will be fractionated by preparative Hg Cs(2)SO(4) density gradient contrifugation. The G-C rich satellite ands will be subjected to restriction endonuclease mapping, and the restriction fragments isolated by preparative electrophoresis and RPC-5 HPLC. These fragments will be amplified by cloning with pBR322 as a vehicle, and subcloned to separate fragments of unique sequence in the divergent population. Both cloned and subcloned fragments will be

analyzed for the extent of sequence divergence and age by the method of high resolution thermal dispersion analysis. Subcloned fragments will be sequenced by the Maxim & Gilbert method.

Progress: 86/10 to 87/09. Bovine satellite I DNA has been purified to >90%, and satellite II to >60%. Clones of pBR322 recombinants of these satellites have been obtained. A high resolution derivative melting curve of the 1402 bp satellite I DNA corresponds perfectly in both temperature and breadth with a large subtransition in the melting profile for total genomic CT-DNA. From the relative integrated areas under the satellite and total CT-DNA melting curves, we estimate this satellite to contribute 15% to the total DNA mass of the bovine genome, which is 2 times more than previous estimates. This translates to a frequency of almost 500,000 coies of the 1402 bp repeat in the genome. A theoretical melting curve for satellite I DNA, produced from the published sequence of a single clone is narrower, but occurs at almost the same T(m). The half band width for the theoretical curve computed from a specific sequence is only 0.180 C, whereas the half band width for the population is 0.580 . The curve for the population of satellite I sequences is symmetrical and broader by 0.367 due to the sequence divergence that has accrued over evolutionary time in an apparent random manner throughout the population. The base composition and standard deviation of the DNA producing the large subtransition is 0.584 +/- 0.0089. We have estimated that this subtransition is produced from the dissociation of a segment of DNA amounting to differece 72% of satellite I.

Publications: 86/10 to 87/09

BLAKE, R.D. 1987. "Cooperative Lengths of DNA During Melting", Biopolymers 26, 1063-1074.

BLAKE, R.D. and EARLEY, S. 1986. "Distribution and Evolution of Sequence Characteristics in the E.coli Genome", J. Biomol. Structure and Dynamics 4, 291-307.

QURESHI, S. and BLAKE, R.D. 1987. "Repetitive Sequences in the Bovine Genome", Me.Biomed.Sci.Symp.

BLAKE, R.D. and DELCOURT, S.G. 1987. "Loop Energy in DNA" Biopolymers 27,001-015.

BLAKE, R.D. and HELEK-DAY, S. 1986. "Parameters in the Theoretical Model for Nonhelical DNA Structures", Macromolecules-86 (Ed. R. Epton), Oxford, p. 143.

BLAKE, R.D. and DELCOURT, S.G. 1987. "Elasticity of DNA in Nonhelical Loops", Fed. Proc. 46,1960.

BLAKE, R.D., HINDS, P.W., EARLEY, S., HILLYARD, A.L. and DAY, G.R. 1986. "Evolution and Functional Significance of the Bias in Codon Usage", in Biomolecular Stereodynamics IV (Eds. R.H. Sarama and M.H. Sarma), Adenine Press, pp. 271-286.

31.023 CRIS0096291

THE PRODUCTION OF CHIMERIC BOVINE IMMUNOGLOBIN GENES

OSBORNE B A; Veterinary & Animal Science; University of Massachusetts, Amherst, **MASSACHUSETTS** 01003.

Proj. No.: MAS00601 Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 85 to 30 SEP 90

Objectives: The availability of monoclonal antibodies has made immunotherapy feasible for disease control and prevention. However, repeated administration of a non-host immunoglobulin can result in hypersensitivity reactions or neutralization of the "foreign" molecule. Host immunoglobulin would be ideal for immunotherapy, since it would function better with the host's effector system and be less immunogenic. But in animals of veterinary interest, the desired antigen specificities are difficult to obtain. In murine systems, however, almost any desired antigen specificity is easy to obtain. A chimeric immunoglobulin molecule consisting of a bovine constant region and a murine variable region would match the effector functions of the molecule to the host and provide the virtually limitless specificities obtainable from the murine system.

Approach: The approach will include cloning bovine immunoglobulin genes, determining their DNA sequence and utilizing cloned murine variable region genes of known antigen binding specificity to create chimeric bovine-murine immunoglobulin molecules possessing the antigen specificity of the murine variable region and having the effector function of the bovine gene.

Progress: 87/10 to 88/09. During the past year we have isolated a number of immunoglobulin genes from a bovine spleen cDNA library. We have isolated a full length mu cDNA and six lambda light chain genes. We also have isolated two genomic mu clones. The DNA sequence of these clones has been determined. Additionally, these clones have been used for probes in Southern blot analysis. A number of interesting findings have resulted from this work. We have shown that unlike the mouse, human, rabbit and rat, the cow appears to possess only one or at most two C lambda genes. All six of our lambda cDNA clones have an identical sequence indicating they represent the same gene. Southern blot analysis also indicate that the C lambda gene is most probably a single copy gene. The bovine C mu is quite similar to its counterpart in mouse and man except that it appears to be 2-300 bp longer than either the human or mouse sequence. It is not clear at present whether these extra sequences constitute a new domain or whether the CH3 or CH4 domain is twice as long as the mouse or human CH3 or CH4 domains. Further sequencing is being done to answer this question. One manuscript is in preparation on the DNA sequences of both the C lambda and the C mu genes. Lastly, we are in the process of isolating the bovine C kappa gene. We intend to use the lambda and the kappa genes in studies to determine the order of rearrangement of bovine light chains.

Publications: 87/10 to 88/09
NO PUBLICATIONS REPORTED THIS PERIOD.

31.024 CRIS0060250
APPLICATION OF GENETIC ENGINEERING TECHNIQUES FOR DAIRY STARTER CULTURE IMPROVEMENT

MCKAY L L; Food Science & Nutrition;
University of Minnesota, St Paul, **MINNESOTA** 55108.
Proj. No.: MIN-18-062 Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 86 to 30 SEP 91

Objectives: To continue screening strains of dairy streptococci for presence of plasmid DNA and attempt to identify metabolic properties that are plasmid mediated. To determine restriction digestion patterns of pertinent plasmids and to identify plasmids that may serve as potential cloning vehicles. To continue characterizing conjugal transfer systems in dairy streptococci and to determine whether chromosomal gene transfer occurs. To begin using the developed plasmid transformation system for strain constructions.

Approach: Phenotypic evidence for plasmid-linked traits will be noted by spontaneous or induced loss of metabolic properties. Physical evidence will be obtained by comparing plasmid profiles of parental and variant strains. Genetic evidence will be obtained by transfer of the plasmid. For isolation of cloning vectors, individual plasmids will be digested with a variety of restriction enzymes. The fragments generated will be separated by agarose gel electrophoresis. Restriction analysis and hybridization studies will reveal similarities between recombinant conjugative plasmids and the chromosome or plasmids in the parental donor. Recombinant DNA techniques will continued to be used to identify, isolate, and characterize the genes responsible for commercially relevant properties in dairy streptococci.

Progress: 88/01 to 88/12. Application of biotechnology to lactic acid bacteria involved in dairy fermentations will rely on development of plasmid biology and genetic systems. The nisin resistance gene and an origin of replication on a plasmid of 60 kb was cloned on a 7.6 kb EcoRI fragment. When self-ligated the fragment existed as an independent replicon and contained a 2.6 kb EcoRI-XbaI region encoding nisin resistance. This replicon may serve as a food-grade cloning vector. Further characterization of the beta-galactosidase gene from S. thermophilus by subcloning and deletion analysis localized it to a 3.2 kb region. The gene was sequenced, which will promote its use in developing cloning vectors and in constructing strains which overproduce the enzyme for food-related applications. The mechanism of reduced bacteriophage sensitivity (Rbs) in Lactococcus lactis KR5 was linked to a single plasmid of about 40 kb which coded an R/M system and a system that suppressed phage development as revealed by reduced plaque size. Previously reported Lac and Suc transconjugants of L. lactis and L. cremoris were examined for sensitivity to prolate and small isometric-headed phage. Four were resistant to small isometric phage and exhibited a reduced plaque size for prolate phage. Study supports observations that Rbs and conjugal transfer ability are physically linked in some lactococci. Results have implications in identifying plasmids coding for Rbs and may aid in explaining dissemination of Rbs genes among lactococci.

Publications: 88/01 to 88/12
FROSETH, B.R., HARLANDER, S.K. and MCKAY, L.L. 1988. Plasmid-mediated reduced phage sensitivity in Streptococcus lactis KR5. J. Dairy Sci. 71:275-284.
FROSETH, B.R., HERMAN, R.E. and MCKAY, L.L. 1988. Cloning of nisin resistance and replication origin on 7.6 kilobase EcoRI fragment of pNP40 from Streptococcus lactis subsp. diacetylactis DRC3. Appl. Environ. Microbiol. 54:2136-2139.
MURPHY, M.C., STEELE, J.L., DALY, C. and MCKAY, L.L. 1988. Concomitant conjugal transfer of reduced bacteriophage sensitivity mechanisms with lactose and sucrose fermenting ability in lactic streptococci. Appl. Environ. Microbiol.
MCKAY, L.L. 1988. Genetic analysis of conjugation in lactic streptococci. J. Dairy Sci. 71 (Suppl. 1):104.
SCHROEDER, C.J. and MCKAY, L.L. 1988. Deletion analysis of the beta-galactosidase gene from Streptococcus thermophilus. J. Dairy Sci. 71 (Suppl. 1):84.

31.025* CRIS0094364
BIOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF BOVID HERPESVIRUS TYPE 4 (BHV-4) ISOLATES FROM CATTLE

HENRY B; School of Veterinary Medicine;
University of Nevada, Reno, **NEVADA** 89557.
Proj. No.: NEV00804 Project Type: HATCH
Agency ID: CSRS Period: 06 AUG 84 to 30 JUN 87

Objectives: Determine the growth characteristics of a number of BHV-4 isolates. Analyze the genome of each. Identify the primary target cells of BHV-4. Determine the transforming potential of BHV-4. Determine the latency of the virus. Determine the presence of absence of BHV-4 defective interfering particles. Determine the degree of genetic relatedness of BHV-4 to other herpesviruses.

Approach: BHV-4 virus isolates will be grown in tissue culture, purified and analyzed by restriction enzymes. Cells of lymphoid origin will be infected and examined for virus replication in particular cell types. Transformation of lymphoid tissue will be attempted using cells from whole blood culture in RPMI media. Virus persistence will be determined by cocultivation with known virus permissive cells.

Progress: 84/08 to 87/06. Our earlier work has focused on the genomic structure and genetic relatedness of Bovine Herpesvirus Type 4 (BHV-4). In addition to published work we are in the process of cloning and mapping the DNA

of BHV-4. However, recently work in our laboratory has centered on determination of the size and number of the polypeptides present in the bovid herpesvirus type 4 (BHV-4) virion. Polyacrylamide gel electrophoresis (PAGE) assays revealed at least 33 proteins, ranging in molecular weight (MW) from 195 to 11.7 kilodaltons (Kd), were present in the purified virion of the BHV-4 prototype strain DN-599. Several of the products were present in relatively large quantity and probably represent virus structural components. One of these proteins, exhibiting an apparent MW of 155 Kd, was presumed to be the main BHV-4 structural polypeptide. Similar studies of two abortion associated virus field isolates revealed protein patterns essentially identical to DN-599. Infection of Madin-Darby bovine kidney (MDBK) cells with BHV-4 was found to significantly alter the cellular protein pattern when compared to that of control uninfected cells. PAGE analysis revealed that 9 proteins were newly synthesized or increased in relative amount while 5 proteins decreased in abundance. Additional studies were performed on extracts of cells at 1 to 8 days post-infection.

Publications: 84/08 to 87/06

EVERMANN, J.F. and HENRY, B.E. (1987). Herpetic infections of cattle: Comparative clinical and diagnostic features of bovine cytomegalovirus and infectious bovine rhinotracheitis. Comp. Cont. Educ. Pract. Vet. (In press).

HENRY, B.E., OTA, R. and EVERMANN, J.F. (1986). The genetic relatedness of disease associated field isolates of bovine herpesvirus type 4 (BHV-4). Amer. J. Vet. Res. 47:2242-2246.

EVERMANN, J.F., KENNEDY, T., CHEEVERS, P., HENRY, B.E. and BARRETT, D. (1985). Diagnostic applications of molecular epidemiology to bovine herpetic infections. 28th Annual Conference of Animal Laboratory Diagnosticians, Milwaukee, WI.

EVERMANN, J.F., HENRY, B.E., KENNEDY, T. and CHEEVERS, P. (1986). Differentiation of bovid herpesviruses of type 1 and 4 by restriction enzyme analysis. IV: Int. Sym. Vet. Lab. Diag., Amsterdam, The FNetherlands.

HENRY, B.E., OTA, R. and EVERMANN, J.F. (1986). Initial characterization of bovid herpesvirus type 4 (BHV-4) proteins. VII: West. Con. Food Animal Vet. Med. Tucson, AR.

31.026 CRIS0132688
INHIBITION OF STREPTOCOCCUS LACTIS BACTERIOPHAGE BY ANTISENSE MRNA

BATT C A; Food Science; Cornell University, Ithaca, **NEW YORK** 14853.
Proj. No.: NYC-143322 Project Type: CRGO
Agency ID: CRGO Period: O1 AUG 87 to 31 JUL 89

Objectives: Proj. 8701467. This project will evaluate the ability of antisense m-RNA to inhibit the lytic cycle of a Streptococcus lactis bacteriophage.

Approach: A gene coding for a bacteriophage structural protein will serve as the target for testing this system. We have recently cloned and characterized a DNA fragment from S. lactis bacteriophage 4-1 which codes for at least three structural proteins. The DNA sequence for this fragment will be determined to locate the 5' end of the coding sequence. The antisense DNA strand corresponding to this 5' end will then be expressed using a native S. lactis promoter to produce an antisense m-RNA. The ability of this antisense m-RNA to inhibit bacteriophage infection will then be tested by challenging a S. lactis strain carrying plasmid borne antisense construction with lytic bacteriophage 4-1.

Progress: 87/01 to 87/12. Loss of starter cultures due to bacteriophage infection remains an important economic problem in the food industry. All previous attempts to control bacteriophage contamination have been developed without any understanding of the bacteriophage's genome structure or its host requirements for replication. We have identified the genes encoding for bacteriophage structural proteins by constructing a S. lactis bacteriophage genomic bank and screened it with polyclonal antibodies against the bacteriophage. A 3 kb region coding for two structural coat proteins has been sequenced completely and open reading frames for the corresponding proteins defined. Once the 5'end of an essential gene is identified it will be transcribed in the reverse orientation to generate antisense mRNA in S. lactis. This antisense mRNA will be used to block bacteriophage infection in vitro.

Publications: 87/01 to 87/12
NO PUBLICATIONS REPORTED THIS PERIOD.

31.027 CRIS0138317
EXPRESSION AND MODIFICATION OF BOVINE BETA-LACTOGLOBULIN

BATT C A; Food Science; Cornell University, Ithaca, **NEW YORK** 14853.
Proj. No.: NYC-143307 Project Type: STATE
Agency ID: SAES Period: O1 JUN 89 to 30 SEP 92

Objectives: The objective of this program is to modify the structure of beta-lactoglobulin and evaluate expression.

Approach: The specific objectives of the proposed program are as follows: To sequence the gene coding for bovine beta-lactoglobulin which has already been isolated. To change the Cys-121 residue in the beta-lactoglobulin gene which has been implicated in the thermal polymerization of the protein. To introduce the parental and mutant forms of the beta-lactoglobulin gene into transgenic mice and determine its expression. To characterize the functionality of beta-lactoglobulin and determine the effect of amino acid substitutions for the Cys-121 residue on thermal polymerization.

31.028* CRIS0045857
FOOT-AND-MOUTH GENE AND ANTIGENIC STRUCTURE: EXPRESSION OF FMDV IMMUNOGENS

MOORE D M; GRUBMAN M J; KENDALL J;
Agricultural Research Service; Plum Island Animal Dis Center, Orient Point, **NEW YORK** 11944.
Proj. No.: 1940-20460-045-00D
Project Type: INHOUSE
Agency ID: ARS Period: 21 NOV 79 to 30 SEP 86

Objectives: To determine the structure (sequence) of immunogens of FMDV and determine the basis for the immune response to FMDV. To compare the variability of FMDV strains through analysis of the genome products and functions. To study and develop protein vaccines produced by chemical synthesis, through gene expression in procaryotes, eucaryotes, and in infectious virusvectors.

Approach: Identify the polypeptide sequences of virus structural and other viral encoded proteins through nucleotide sequencing of cloned viral genetic material or directly sequence selected polypeptides. Compare sequences of variants to study variation in antigenicity of FMDV. Study the basis of immunization through the preparation of experimental vaccines using poly- peptides generated by chemical synthesis, or biosynthesis in cells engineered to produce viral proteins. Immunize laboratory animals and livestock to determine immunization and protection against FMD with variouspolypeptide vaccines. Identify the location and structure of antigenic sites through competitive inhibition using selected synthetic peptides. Explore the feasibility of constructing native viral antigenic structures as vaccines through expression of viral genes in transformed cells or by viral vectored FMD genes. Study the processing of viral antigens through selective cloning techniques.--Plum Island, NY, Molec. Biol. Lab. 101-C, BL-3, 1/29/80, DM Moore/DO Morgan/MJ Grubman/JL Card/M Zellner/KH Axelson.

Progress: 86/01 to 86/12. Gene segments coding for FMDV viral protein VP-1 cloned into bacterial plasmid expression vectors synthesize high levels of the polypeptide in E. coli. Type A12 VP-1 has been extensively tested in cattle to evaluate the effectiveness of the protein vaccine. High levels of immunity were obtained in the majority of animals and reduced severity of symptoms was observed for animals which became infected on challenge of immunity. Virus type O1 VP-1 has previously generated poor immunity in livestock. Recent tests with a combined polypeptide of two areas of the VP-1 protein generated moderate protective levels in cattle vaccinated twice. In connection with antigenic analysis of VP-1 of type A and O FMDV, variants resistant to specific neutralizing monoclonal antibodies were generated. The variants are analyzed for nucleotide sequence changes in the RNA genome to pinpoint the important sites relating to immunization. The gene segment for VP-1 has been isolated and engineered into vaccinia virus as an experimental, live recombinant viral vaccine. Cell cultures infected with the recombinant virus synthesize FMDV VP-1, but vaccination of guinea pigs, cattle and mice failed to mount an antiviral response. It is likely that the cytoplasmically located VP-1 antigen was not released or that the immunogenicity of the VP-1 as presented to the host was poor. Experiments are being extended to incorporate additional structural components to facilitate morphogenesis and increase potency.

Publications: 86/01 to 86/12
NO PUBLICATIONS REPORTED THIS PERIOD.

31.029* CRIS0140880
PRIMARY STRUCTURE OF THE FMDV GENOME AND GENERATION OF INFECTIOUS DNA CLONES

MOORE D M; WIMMER E; VAKHARIA V; Microbiology; State University of New York, Stony Brook, **NEW YORK** 11794.
Proj. No.: 1940-34000-012-04S
Project Type: COOPERATIVE AGREE.
Agency ID: ARS Period: 01 OCT 85 to 30 SEP 87

Objectives: To develop rapid methods to sequence and study the primary structure of theFMDV genome to determine function of the genome and antigenic characteristics of capsid protein antigens. To examine the transcription and translation in vitro and the expression of cDNA segments of FMDV protein coding regions in tissue cultures. To study the processing of FMDV polyproteins and evaluate the possibility of generating native structures of the capsid proteins of the virus.

Approach: Sequence data will be obtained from the genomic RNA or cloned cDNA segmentsof the FMDV genome. Specific areas of the genome will be selected for study, with the non-coding areas of the genome, the capsid protein coding regions, and coding regions for other non-structural proteins. Specialized vectors containing protein coding cDNA segments of the FMDV genome will be transcribed in vitro and subsequently translated in vitro. Vectors will be transfected into mammalian tissue culture cells to study the expression/ assembly of the capsid region. The function of non-structural proteins and the antigenicity of capsid structural proteins will be studied in this manner. The regeneration of progeny virus from cloned cDNA will be considered by transfection of either plasmids containing full-length genomeinserts or transfection of RNA transcripts of cDNA clones.

Progress: 88/01 to 88/12. Previously, in vitro transcription/translation systems were used to determine the requirements for expression and proteolytic processing of the capsid polyprotein precursor molecule into the individual capsid proteins. To examine the processing of FMDV proteins expressed in vivo, two transient expression systems were developed. Different segments of the coding sequence of the FMDV genome were cloned into plasmids containing either the bacteriophage T7 promoter or a vaccinia late promoter. These were transfected into tissue culture cells infected with a recombinant vaccinia virus expressing the T7 RNA polymerase or with wild

type vaccinia virus WR, respectively. Cells were harvested and extracts of the cells were analyzed by western blot analysis with a VP1 antiserum/125I-protein A detection system. The results showed that the P1-2A region was efficiently cleaved from precursor polyprotein and that the P1 region was further processed to capsid polypeptides if the clones contained the coding sequence for the viral protease, 3C. The results indicate that FMDV proteins expressed under the control of the T7 promoter or a vaccinia promoter can be effectively processed into capsid proteins in vivo and that stable vaccinia virus recombinants should be able to be engineered to express the same proteins. Work is underway to engineer and isolate such recombinant vaccinia viruses.

Publications: 88/01 to 88/12

VAKHARIA, V.N., DEVANEY, M.A., GRUBMAN, M.J., and MOORE, D.M. 1988. Cloning and expression of foot-and-mouth disease virus genes. XI Pan American Congress of Veterinary Sciences. Lima, Peru. (Abstract).

31.030 CRIS0133467

MOLECULAR CHARACTERIZATION OF PTR2030-DIRECTED PHAGE RESISTENCE IN LACTIC STREPTOCOCCI

KLAENHAMMER T R; Food Science; North Carolina State University, Raleigh, **NORTH CAROLINA** 27695.

Proj. No.: NC09350 Project Type: CRGO
Agency ID: CRGO Period: 01 SEP 87 to 31 AUG 90

Objectives: Proj. 8701584. Develop phage resistant starter cultures for dairy fermentations through genetic strategies that assemble complementary gene-directed mechanisms which inhibit bacteriophage infection and proliferation.

Approach: Localize, define and characterize genetic determinants and specific genes or gene sequences responsible for pTR2030-encoded phage resistance. Cloning and expression of the responsible pTR2030 genes or gene sequence on plasmid vectors transmissible in lactic streptococci. Definition of the mechanism(s) of pTR2030-directed phage resistance and genetic characterization of phages susceptible or resistant to inhibition by pTR2030. Construct and evaluate gene combinations in lactic streptococci that assemble phage defenses complementary to pTR2030-directed resistance.

Progress: 88/01 to 88/12. The plasmid pTR2030 encodes phage resistance (Hsp and R/M) in Lactococcus lactis. Molecular characterization has identified a 13.6 kb region that encodes genetic determinants for phage resistance. This region has been cloned into the E. coli - Lactococcus shuttle vector, pSA3, to form the recombinant plasmid pTK6. pTK6 has been characterized by deletion mapping and transposition mutagenesis. Mutagenesis with Tn5 localized a 3 kb region which is essential for expression of Hsp. Any of four Tn5 insertions within the 3 kb region eliminated Hsp, whereas 26 insertions outside the locus had no effect on the phage resistance phenotype. Deletion analysis across the 13.6 kb region confirmed that the 3 kb region contained all the information necessary for expression of Hsp. An in vivo deletion in pTK6, combined with definition of the 3 kb hsp locus, provided physical evidence for a second defense mechanism located within the 13.6 kb fragment from pTR2030. Genetic determinants for R/M were localized and segregated from hsp genes. Molecular characterization of the cloned 13.6. kb region from pTR2030 has identified an insertion sequence and genetic loci for two distinct phage defense mechanisms (R/M and Hsp) which contribute to the phage resistance imposed by pTR2030.

Publications: 88/01 to 88/12

KLAENHAMMER, T.R. 1988. Genetic characterization of multiple mechanisms of phage defense from a prototype phage insensitive strain, Lactobacillus lactis ME2. J. Dairy Sci. (Supplement) 71:105 (Abstract, D129).

ROMERO, D.A. and KLAENHAMMER, T.R. 1988. Phage resistance directed by hsp determinants from pTR2030 is enhanced by genetic recombination with conjugal plasmids in lactococci. J. Dairy Sci. (Supplement) 71:62 (Abstract, D7).

31.031 CRIS0135994

MOLECULAR CHARACTERIZATION OF GENES ENCODING PHAGE RESISTANCE IN LACTIC ACID BACTERIA

KLAENHAMMER T R; Food Science; North Carolina State University, Raleigh, **NORTH CAROLINA** 27695.

Proj. No.: NC02168 Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 88 to 30 SEP 93

Objectives: Develop phage-insensitive cultures for dairy fermentations through genetic strategies to transfer, combine, and express complementary restriction and modification (R/M) systems in lactic acid bacteria.

Approach: Localize, define, and clone and the genetic determinants and specific r/m genes or gene sequences responsible for phage restriction and modification activities in lactic streptococci. Characterize structural and regulatory gene sequences, define r/m gene products, and investigate factors affecting expression of R/M . Construct gene combinations in lactic acid bacteria which assemble different R/M systems and evaluate the activity of r/m components with each other as well as in combination with other phage defense mechanisms.

31.032* CRIS0133670
DEVELOPMENT OF BHV1 AS A BOVINE RESPIRATORY VACCINE VECTOR

EBERLE R; D'OFFAY J M; FULTON R W; Veterinary Medicine; Oklahoma State University, Stillwater, **OKLAHOMA** 74078.
Proj. No.: OKL02034 Project Type: ANIMAL HEALTH
Agency ID: CSRS Period: 01 JAN 88 to 30 SEP 93

Objectives: Construct a BHV1 strain as a vaccine vector. Delete the DNA sequences coding for a non-essential glycoprotein of BHV1 and determine the properties of the gene-deletion virus in vitro and in vivo.

Approach: Locate the BHV1 glycoprotein gene using a plasmid containing a cDNA from the related PRV gIII glycoprotein. Clone and sequence the gene and adjacent non-coding sequences. Delete the gIII coding sequences by hybridization of synthetic oligodeoxynucleotide to single-stranded, cloned DNA in M13 vector. Incorporate the gIII gene deletion into BHV1. The replicative ability of the gene virus will be quantitatively assessed, and its failure to express any polypeptides antigenically related to gIII confirmed. Test the immune response to the gIII virus in rabbits.

31.033 CRIS0132800
"ISOLATION AND CLONING OF THE (R)-LACTYL-COA DEHYDRATASE GENE FROM MEGASPHAERA ELSDENII"

SANDS J A; PHILLIPS J A; Lehigh University, Bethlehem, **PENNSYLVANIA** 18015.
Proj. No.: PENR-8701161 Project Type: CRGO
Agency ID: CRGO Period: 01 SEP 87 to 31 AUG 89

Objectives: Proj. 8701161. The overall objective of this proposal is to isolate the (R)-lactyl-CoA dehydratase gene from Megasphaera elsdenii and clone it into Lactobacillus lactis.

Approach: Isolate and purify the (R)-lactyl-CoA dehydratase. Determine the amino acid sequence of the purified protein and synthesize DNA probes from the amino acid sequence. Construct and screen the genomic bank of M. elsdenii with the DNA probes for the (R)-lactyl-CoA dehydratase gene. Fuse the isolated gene downstream of the lac promoter and insert the lac promoter (R)-lactyl CoA dehydratase fusion into a Lactobacillus cloning vector. Screen Lactobacillus lactis for expression of the (R)-lactyl-CoA dehydratase gene.

31.034 CRIS0095550
ATTENUATED SALMONELLA VACCINE

BENSON C; Clinical Studies; New Bolton Center, Kennett Square, **PENNSYLVANIA** 19348.
Proj. No.: PENV-5-20383 Project Type: STATE
Agency ID: CSVM Period: 15 DEC 83 to 30 SEP 88

Objectives: The generation of attenuated strains of Salmonella which may be utilized to immunize horses and food animals by the oral route.

Approach: Several isolates from clinical cases of Salmonellosis will be attenuated by selective transposon insertion and deletion (Bochner technique). Attenuation will be assessed by an in vitro procedure and a mouse protection:challenge system prior to trial immunization in the equine and bovine species. The presence of an enterotoxin gene in Salmonella will be determined by gene hybridization analysis utilizing several different genetic probes. The enterotoxin gene will be isolated and the nucleotide sequence varied to produce a toxoid. The attenuated gene will be inserted into the genome of vaccine strains by transposon insertion.

Progress: 88/01 to 88/12. An attenuated vaccine of Salmonella group B has been developed and tested extensively in mice. One dose (10 cfu/dose) administered per os to Balb/c mice was sufficient to protect against a 10 MLD of a virulent isogenic strain. Two vaccine doses, per os at 2 week intervals, stimulated a sufficient level of cross-immunity to protect against a 10 MLD dose of a highly virulent strain of S. enteritidis. This vaccine is intended for use in bovine, equine and avian species.

Publications: 88/01 to 88/12
NO PUBLICATIONS REPORTED THIS PERIOD.

31.035* CRIS0132970
MOLECULAR AND SOMATIC CELL GENETICS: MAPPING THE CATTLE GENOME

WOMACK J E; Veterinary Pathology; Pb Box 3578, College Station, **TEXAS** 77843.
Proj. No.: TEX06912 Project Type: CRGO
Agency ID: CRGO Period: 01 SEP 87 to 31 AUG 90

Objectives: To map cattle genes of key physiological significance, including the genes coding immunoglobulins, prolactin, parathyroid hormone, fibronectin, homeotic boxes & interferons. To determine the extent and nature of variation in these genes in breeding populations of cattle. To determine the molecular organization and natural genetic variability of the interferon, immunoglobulin, and MHC multigene families. Proj. 8701679.

Approach: The combined technologies of somatic cell and molecular genetics will be utilized to produce both a genetic and a physical map of the cattle genome. A large pedigreed herd will be tested for restriction fragment length polymorphisms (RFLPs) to evaluate the genetic diversity in key genes & gene families and to establish a genetic (recombination) map.

Progress: 88/01 to 88/12. The objectives of this project are to utilize somatic cell genetic methods to generate a gene map of the cow and to expand this map by isozyme, DNA fragment, and karyotypic analysis. Restriction fragment length polymorphisms are being

discovered that will permit the combination of recombinant mapping in meiosis with synteny mapping in somatic cells. The ultimate objective is to utilize the map to define markers of disease resistance and productivity. A total of 12 DNA polymorphisms have been discovered, including genes for osteonectin, gamma crystallin, and 21-steroid hycroxylase (not reported previously). Each of these has been added to the synteny map by analysis of hybrid somatic cells. The total number of genes mapped in cattle is now over 100 with 8 assignments of syntenic groups to specific chromosomes.

Publications: 88/01 to 88/12

WOMACK, J.E. Genetic engineering in agriculture: animal genetics and development. Trends in genetics 3:65-68, 1987.

WOMACK, J.E. A gene map of the cow. Genetics Maps 4:499-501, 1987.

ADKISON, L.R., LEUNG, D.W. and WOMACK, J.E. Somatic cell mapping and restriction fragment analysis of bovine alpha and beta interferon gene families. Cytogenet. and Cell Genet. 47:62-65, 1988.

WOMACK, J.E. Comparative gene mapping: A valuable new tool for mammalian developmental studies. Developmental Genet. 8:281-293, 1987.

WOMACK, J.E. Molecular cytogenetics of cattle: a genomic approach to disease resistance and productivity. J. Dairy Sci. 71:1116-1123, 1988.

MCAVIN, J.C., PATTERSON, D. and WOMACK, J.E. Mapping the bovine PRGS and PAIS genes in hybrid somatic cells: syntenic conservation with human chromosome 21. Biochem. Genet. 26:9-18, 1988.

SKOW, I.C., WOMACK, J.E., PETRASH, J.M. and MILLER, W.L. Synteny mapping of bovine genes for 21 steroid hydroxylase, alpha A-crystallin and class I bovine leucocyte antigen (BoLA) in cattle. DNA 7:143-149, 1988.

31.036* CRIS0097956

MOLECULAR GENETICS OF CYTOCHROME P-450 GENE FAMILIES IN CATTLE

SKOW L C; Veterinary Anatomy; Texas A&M University, College Station, **TEXAS** 77843.

Proj. No.: TEX06831 Project Type: ANIMAL HEALTH
Agency ID: CSRS Period: 14 FEB 86 to 30 SEP 88

Objectives: To establish the chromosomal locations of four families of cytochromes P-450 in cattle and identify restriction endonuclease fragment polymorphisms in cytochrome P-450 genes among eight breeds of cattle for use as gene markers for P-450 genotypes in subsequent experiments to correlate P-450 genotypes with levels of xenobiotic metabolism.

Approach: Bovine cytochrome P-450 genes will be assigned to chromosomes by DNA hybridization analysis of bovine x hamster somatic cell hybrid clones. Genetic variation in bovine cytochrome P-450 genes will be detected as restriction fragment length polymorphisms in DNA prepared from lymphocytes or semen collected from an experimental cattle herd maintained by the Texas Agricultural Experiment Station (H6749).

Progress: 86/01 to 88/09. The objective of this project is: to define genetic markers for various genes of the cytochrome P-450 monoxygenase system; to better understand the organization and distribution of these genes in the bovine genome; and, to develop genetic systems to search for cytochrome P450-mediated toxicity. Clones of seven genes have been acquired as cDNAs, including: 11 beta, 15 yield, 17 yield, and 21 steroid hydroxylases, adrenodoxin, side chain cleavage enzyme, and the phenobarbital and methylcholanthrene-inducible forms of P-450. We have identified the chromosomal location of 21OH and defined RFLPs for 21OH, 17 yield and SSC in cattle. These studies will continue as planned until each of the P-450 genes identified abouve has been genetically characterized in cattle.

Publications: 86/01 to 88/09

SKOW, L.C., WOMACK, J.E., PETRESH, J.M. and MILLER, W.C. 1988. The genes for 21 steriod hydroxylase and (d)A-crystalline are not syntenic in cattle. DNA. 7:143-149.

31.037* CRIS0135643

MOLECULAR GENETICS AND MAPPING OF DISEASE RESISTANCE GENES IN CATTLE

SKOW L C; Veterinary Anatomy; Texas A&M University, College Station, **TEXAS** 77843.

Proj. No.: TEX06923 Project Type: HATCH
Agency ID: CSRS Period: 22 AUG 88 to 31 JUL 93

Objectives: 1. Establish the chromosomal locations of loci for the major histocompatibility complex, macrophage function, and various forms of cytochrome P-450 monooxygenases in cattle. 2. Identify genetic polymorphisms among cattle that may be correlated with predisposition to diseases.

Approach: Recombinant DNA techniques will be used to analyze somatic cell hybrid clones from bovinexhamster cell fusions to assign bovine genes to chromosomes. Clones of different genes from humans, mice or cattle will be 32P-labelled and hybridized to DNA from somatic cell hybrid cell lines that have randomly lost cattle chromosomes. The concordant presence or absence of gene sequences with specific chromosomes will be used to assign genes to cattle chromosomes. Intraspecies variation in disease resistance genes will be detected by analysis of DNA from pedigreed cattle in multibreed herds supported by TAES (H6749). DNA will be analyzed by restriction endonucleases and blot hybridization with radioactive gene probes to detect restriction fragment length polymorphisms (RFLP).

Progress: 88/01 to 88/12. A major research objective in animal agriculture is the development of genetic approaches to increase disease resistance in cattle. Several bovine genes and gene families have been targeted for analysis based on reported disease association

of homologous genes in other species, notably mice and humans. Genes being studied include the major histocompatibility complex (BoLA), several genes of the cytochrome P-450 mono-oxygenase system, gamma crystallin and fibronectin. The latter two genes are closely linked to a gene conferring resistance to a broad range of intracellular pathogens in mice. Restriction fragment length polymorphisms have been identified for each of these genes in cattle and are being used as gene markers to search for disease resistance genes. Recently, we completed genetic analysis of a herd of cattle demonstrating brucellosis resistance. Fifteen gene markers have been developed in this herd of 148 animals, and data are presently being analyzed for correlation with brucellosis resistance.

Publications: 88/01 to 88/12

ADKISON, L., SKOW, L.C., THOMAS. T.L., PETRESH, J.M. AND WOMACK, J.E.: 1988. Somatic cell mapping and restriction fragment analysis of bovine genes for fibronectin and gamma crystallins. Somat. Cell Genetics. 47:155-159.

SKOW, L.C., DONNER, M.E., HUANG, S-M., TAYLOR, B.A., BEAMER, W.G., AND LALEY, P.A.: 1988. Gene sequences for mouse gamma crystallins are on chromosome 1. Biochem. Genet. 26:557-570.

BEAMER, W.G., TENNENT, B.J., SCHULTZ, K.L., NADEAU, J.H., SCHULTZ, N.L. and SKOW, L.C.: 1988. Gene for Ovarian Granulosa Cell Tumor Susceptibility, GCT, in SWXJ Recombinant Inbred Strains of Mice Revealed by Dehydroepiandrosterone.

31.038* CRIS0136233

ORGANIZATION AND EXPRESSION OF BOLA CLASS I GENES

SKOW L C; Veterinary Anatomy; Texas A&M University, College Station, **TEXAS** 77843.
Proj. No.: TEX06958 Project Type: CRGO
Agency ID: CRGO Period: 01 SEP 88 to 31 AUG 91

Objectives: PROJ. 8801608. Define the organization and arrangement of selected genes within the major histocompatibility complex (MHC) of cattle (BoLA). Identify genetic polymorphisms among cattle that may be correlated with predisposition to diseases.

Approach: Pulsed field gel electrophoresis will be used to analyze large (10 bp) DNA fragments in order to establish an extended restriction map of the BoLA region. Organization of BoLA genes can then be compared to the homologous regions in humans and mice to better understand the role of the MHC in disease. Intraspecies variation in disease resistance genes will be detected by analysis of DNA from pedigreed cattle in multibreed herds supported by TAES (H6749). DNA will be analyzed by restriction endonucleases and blot hybridization with radioactive gene probes to detect restriction fragment length polymorphisms (RFLP). RFLPs will be used to determine linkage relationships and to identify genotypes for additional experiments.

Progress: 88/09 to 88/12. The major histocompatibility complex (MCH) consists of a large number of closely-linked genes that encode cell-surface glycoproteins known as histocompatibility antigens. Genes of the MHC are involved in regulation of cell and humoral immune response and variant MHC genes have been associated with a variety of disease susceptibilities in humans and mice. Genetic analysis of the bovine MHC (called BoLA) is an important objective in understanding genetic predisposition to disease in cattle. We have tentatively assigned the BoLA complex to chromosome syntenic group U21 based on linkage studies using the flanking gene markers, glyoxylase and 21 steroid hydroxylase. Eleven clones of BoLA Class I DNA sequences have been isolated and are being characterized to determine gene expression and organization of the bovine MCH complex. Restriction enzyme analysis of bovine genomic DNA has identified a large number of BoLA restriction fragment length polymorphisms which are being used to confirm the chromosome assignment and to further investigate the role of BoLA in cattle diseases.

Publications: 88/09 to 88/12

SKOW, L.C., WOMACK, J.E., PETRESH, J.M. and MILLER, W.C. 1988. The genes for 21 steroid hydroxylas and A-crystallin are not syntenic in cattle. DNA. 7:143-149.

31.039* CRIS0130274

MOLECULAR DIFFERENTIATION OF NONCYTOPATHIC AND CYTOPATHIC ISOLATES OF BOVINE VIRAL DIARRHEA VIRUS

COLLISSON E; KEMP M; SNEED L; Veterinary Microbiology & Parasitology; Texas A&M University, College Station, **TEXAS** 77843.
Proj. No.: TEX06864 Project Type: SPECIAL GRANT
Agency ID: CSRS Period: 01 SEP 86 to 28 FEB 90

Objectives: Cytopathic and noncytopathic isolates of bovine viral diarrhea virus (BVDV) have recently been shown to have synergistic properties in the host. Persistent infection of cattle with noncytopathic BVDV apparently predisposes the animal to clinical, often fatal, mucosal disease after exposure to a cytopathic isolate. It is the overall objective of this proposal to define differences at the molecular level which are responsible for these distinguishing biological properties.

Approach: The structural relationships of the proteins derived from disease producing pairs of cytopathic and noncytopathic BVDV will be determined. Oligonucleotides which may be unique for cytopathic and unique for noncytopathic and those which are conserved for all isolates of BVDV will be identified and sequenced. An oligonucleotide map of the genomes of cytopathic and noncytopathic BVDV will be constructed. The nucleotide sequences which differentiate cytopathic and noncytopathic isolates will be determined.

Progress: 88/01 to 88/12. We have developed a technique to compare the genomes of BVDV strains through fingerprint analyses. The

purified RNA is cut with T1 RNase and the resulting fragments are labelled with 32P-pCp. T1 RNase fingerprint analyses are being completed for several strains of BVDV; including Illinois noncytopathic; cytopathic SSD; cytopathic and noncytopathic TGA, and NADL. The fingerprints have been completed and we are in the process of interpreting the data. The preliminary results of the two TGA strains indicate that the cytopathic and noncytopathic strains isolated from the same animal do not differ genetically since this pair are very similar. The cytopathic SSD is genetically also very close to the TGA whereas the Illinois isolate differs considerably. We are in the process of examining the second strain of the SSD and Illinois pairs to determine the relatedness of several pairs of cytopathic and noneytopathic strains isolated from the same animal. If the pattern is similar to the TGA pairs, it would indicate that the appearance of cytopathic strains in persistently infected animals is the result of a switch of portion of the noncytopathic strain to a cytopathic form. Since in tissue culture these strains retain their characteristic cytopathology, it would seem that such a switch is directed, is a genetically committed situation and is driven by factors not present in cell culture.

Publications: 88/01 to 88/12
NO PUBLICATIONS REPORTED THIS PERIOD.

31.040* CRIS0093813
CLONING AND EXPRESSION OF PROTECTIVE IMMUNOGEN GENES OF BRUCELLA ABORTUS

FICHT T A; ADAMS L C; SOWA B A; Veterinary Microbiology & Parasitology; Texas A&M University, College Station, **TEXAS** 77843.
Proj. No.: TEX06781 Project Type: SPECIAL GRANT
Agency ID: CSRS Period: 01 SEP 84 to 31 DEC 87

Objectives: To isolate the genes encoding outer membrance group 2 and group 3 porius of Brucella abortus. To clone the genes and compare the amino acid sequence of outer membrane group 2 and group 3 porius of virolent and attenuated strains of B. abortus. To establish protective immunity in laboratory animals and cattle by vaccination with recombinant surface immunogeus of B. abortus.

Approach: B. abortus surface immunogeus (group 2 and group 3 perius) will be expressed in E. coli as fusion products of B-galactosidasc. This will be accomplished by the use of the bacteriophage lambda expression vector (lambda gt11).

Progress: 84/09 to 87/12. Brucella abortus DNA (S19 and S2308 fragments of between 300 and 1500 bp) has been successfully cloned and expressed in the lambda gt11 vector system. Recombinants expressing portions of five outer membrane proteins have been purified to homogeniety via selection utilizing antisera raised agaist SDS-PAGE purified proteins prepared from cellular envelopes of a rough mutant of B. abortus. Fusion products of the major outer membrane proteins synthesized under the control of the E. coli lac PO were used to vaccine trials in cattle. Fusion products were purified utilizing an anti-beta-galactosidase column. Twenty-five head of cattle were vaccinated with 3.6 mg each of a cocktail of fusion products in two doses over sixty days representing 30 mu g of brucella antigen. No protection was observed in cattle vaccinated above that experienced by cattle vaccinated with the adjuvant control alone. However, genetic characterization of the recombinants via restriction mapping and DNA sequence analysis has indicated that only a select portion of each gene was fused to beta-galactosidase in the recombinants isolated. As a result, the cattle received a very limited repertoire of outer membrane protein antigens. This may represent a limitation in the lambda gt11 system with regard to the cloning of integral membrane proteins. The B.

Publications: 84/09 to 87/12
FICHT, T.A., BEARDEN, S.W., SOWA, B.A. and ADAMS, L.G. Multiple Copies of a Gene Encoding a Major Outer Membrane Protein of Brucella abortus are Clustered within a Four Kilobase Stretch of Genomic DNA. Infect. and Immun. Submitted for pub.
FICHT, T.A., BEARDEN, S.W. and SOWA, B.A. 1987. DNA sequence of the omp II operon of Brucella abortus. Manuscript in preparation.
FICHT, T.A. and BEARDEN, S.W. 1987. Conservation of the omp II operon in the family Brucellae. Manuscript in preparation.

31.041 0069460
BOVINE BRUCELLOSIS: DIAGNOSIS, VACCINATION IMMUNITY, PATHOGENESIS & EPIDEMIOLOGY

ADAMS L G; CRAWFORD R P; FICHT T A; Veterinary Pathology; Texas A&M University, College Station, **TEXAS** 77843.
Proj. No.: TEX06194 Project Type: HATCH
Agency ID: CSRS Period: 07 MAR 89 to 28 FEB 94

Objectives: (1) To develop and evaluate inactivated and live brucellosis vaccines that do not stimulate antibodies cross-reactive in serologic test, (2) to develop differential and diffinitive assays to diagnose vaccinated or field strain infected cattle, (3) to delineate the genetic basis of the mechanisms of disease pathogenesis and natural resistance, and (4) to identify epidemiologic factors contributing to transmission of Brucella abortus.

Approach: Improved brucellosis vaccines will be approached by: (a) purifying protective immunogens, (b) structural characterization and amino acid sequence determination of immunogens, (c) cloning genes encoding immunogens, and either (d) deleting genes encoding for virulent factors or dominant antigens or inserting protective immunogen genes into expression vectors. Improved differential diagnostics will be approached by (a) isolating strain-specific antigens, (b) developing strain-specific monoclonal antibodies, (c) developing Brucella spp. DNA hybridization probes, (d) or application of

monoclonal antibodies in antigen capture ELISA procedures. The mechanisms of disease pathogenesis and natural disease resistance will be approached by: (a) establishing the heritability of disease resistance in cattle, (b) studying B. abortus adhesins for mucosal receptors (c) identifying the mode of entry, invasion and survival of B. abortus and (d) delineating the role of bovine T-lymphocytes in protective immunity.

Progress: 88/01 to 88/12. The inability to diagnostically differentiate between vaccinated and field infected cattle is the major shortcoming of the current USDA approved strain 19 vaccine. We have isolated carbohydrate and protein components of Brucella abortus cell wall and cloned segments of the genes that encode principal protein components of the cell for evaluation as brucellosis vaccines. We recently demonstrated that our killed subunit cell envelope vaccine from a Brucella abortus rough mutant protected 50% of pregnant cows from brucellosis without causing false positives on USDA diagnostic tests. This vaccine is designed to eliminate the problems of false positive reactions and human infections associated with Strain 19 vaccine. Monoclonal antibodies against carbohydrate and protein antigens of Brucella abortus were pyoduced and used in competitive enzyme-linked immunosorbent assay technologies for detection of antigens and differentiating antibodies stimulated by field strain vs Strain 19 vaccine. Trransposon mutagenesis of B. abortus is being used to generate mutants as vaccine candidates which are being evaluated to confirm their lack of reversion to virulence and their ability to induce protective immunity in small ruminants priior to evaluation in cattle.

Publications: 88/01 to 88/12

OVERHOLT, K. TEMPLETON, J.W. and ADAMS, L.G. Standardization of ELISA Calibration Curves the Biomek trademark 1000 Workstation. Beckman Instruments Biomek Bulletin, pp. 1-2, January 1988.

CRAWFORD, R.P., ADAMS, L.G. and RICHARDSON, B.E. Correlation of field strain exposure and brucellosis incidence in 6 beef herds vaccinated with strain 19. J. Am. Vet. Med. Assoc., 5:191-196, 1988.

BARRIOS, D.R., KRAMER, D.C., BESSOUDO, E. and ADAMS, L.G. Embryo collection and bacteriology in Brucella abortus culture-postive superovulated cows. Theriogenology, 29:(2)353-361, 1988.

CRAWFORD, R.P., ADAMS, L.G., and CHILDERS, A.B. Value of Serologic Reactions at 2 Months Following Strain 19 Vaccination of Cattle Herds with Brucellosis. Prev. Vet. Med. 5:(4)275-280, 1988.

CRAWFORD, R.P., SANDERS, R.B. and ADAMS. L. G. Early Removal of Cows with Brucellosis and the Effect in Strain 19 Vaccinated Cattle Herds. Prev. Vet. Med., 5:191-195, 1988.

DAVIS, D.S., HECK, H.C., WILLIAMS, J.D., SIMPSON, T.R. and ADAMS, L.G. Interspecific transmission of Brucella abortus from experimentally infected coyotes (Canis latrans) to parturient Bos taurus heifers. J. Wildl. Dis. 24(3):533-537, 1988.

HARMON, B.G., ADAM, L.G. and FREY, M. Survival of rough and smooth strains of.

31.042* CRIS0095472
MOLECULAR GENETICS OF CATTLE

WOMACK J E; Veterinary Pathology; Texas A&M University, College Station, **TEXAS** 77843.

Proj. No.: TEX06718 Project Type: HATCH
Agency ID: CSRS Period: 25 APR 85 to 31 MAR 90

Objectives: To generate a genetic map of cattle chromosomes. To identify restriction fragment length polymorphisms associated with cattle health and productivity. To clone cattle genes for gene injection experiments.

Approach: To combine somatic-cell genetics and recombinant DNA technologies to study the organization of the cattle genome at the molecular level. DNA polymorphisms associated with health and productivity will be identified as gene markers for effective breeding programs. Specific genes useful for improvement of cattle by gene transfer protocols will be identified, isolated, and cloned by molecular genetic technology.

Progress: 88/01 to 88/12. The objectives of this project are to utilize somatic cell genetic methods to generate a gene map of the cow and to expand this map by isozyme, DNA fragment, and karyotypic analysis. Restriction fragment length polymorphisms are being discovered that will permit the combination of recombinant mapping in meiosis with synteny mapping is somatic cells. The ultimate objective is to utilize the map to define markers of disease resistance and productivity. We have now mapped over 100 genes to 26 cow chromosomes. A total of 12 DNA polymorphisms have been discovered, including genes for osteonectin, gamma crystallin 21-steroid hydroxylase, beta hemoglobin, and parathyroid hormone (no reported previously). Each of these has been ddded to the synteny map by analysis of hybrid somatic cells.

Publications: 88/01 to 88/12

WOMACK, J.E.: Genetic engineering in agriculture: animal genetics and development. Trends in Genetics 3:65-68, 1987.

WOMACK, J.E. A gene map of the cow. Genetic Maps 4:499-501, 1987.

ADKISON, L.R., LEUNG, D.W., and WOMACK, J.E.: Somatic cell mapping and restriction fragment analysis of bovine alpha and beta interferon gene families. Cytogenet. and Cell Genet. 1987.

WOMACK, J.E.: Comparative gene maping: A valuable new tool for mammalian developmental studies. Developmental Genetics 8:281-293, 1987.

WOMACK, J.E.: Molecular cytogenetics of cattle: a genomic approach to disease resistance and productivity. J. Dairy Sci. 71:111601123, 1988.

MCAVIN, J.C., PATTERSON, D., and WOMACK, J.E.: Mapping the bovine PRGS and PAIS genes in hybrid somatic cells: syntenic conservation with human chromosome 21.

Biochem. Genet. 26:9-18, 1988.
SKOW, L.C. WOMACK, J.E., PETRASH, J.M. and MILLER, W.L.: Synteny mapping of bovine genes for 21 steroid hydroxylase, alpha A-crystallin and class I bovine leucocyte antigen (BoLA) in cattle. DNA 7:143-149, 1988.

31.043* CRIS0099941
GENE MAPPING IN CATTLE BY IN SITU HYBRIDIZATION OF G-BANDED CHROMOSOMES

WOMACK J E; Veterinary Pathology; Texas A&M University, College Station, **TEXAS** 77843.
Proj. No.: TEX06867 Project Type: CRGO
Agency ID: CRGO Period: 01 SEP 86 to 31 AUG 89

Objectives: PROJECT 8601309. To map bovine casein, growth hormone, BOLA, 21-OH, and interferon genes to chromosomes. To map these bovine genes to specific chromosomal sites. To develop probes for the in situ mapping of other bovine genes.

Approach: To hybridize radio-labeled probes to cattle chromosomes in situ in order to determine the precise chromosomal location of physiologically significant genes and gene families.

Progress: 88/01 to 88/12. The objectives of this proposal are to utilize in situ hybridization of G-banded chromosomes to physically map genes that are being genetically mapped by other methods. These include interferons, caseins, and several x-linked genes. We have developed methods to stain cattle chromosomes, both by G- and Q- banding, in conjunction with hybridization to labeled probes and autoradiography. Phosphoglycerate kinase-1 clotting factor IX, and the region homologous to human Duchenne muscular dystrophy have been localized on the X chromosome. Probes of high specificity for interferon, fibronectin, gamma crystallin, and caseins have been developed. By using oligolabeling of probes rather than nick-translation, we have been able to use H nucleotides rather than I, and significantly reduce a potential health hazard in the laboratory. The fibronectin and gamma crystallin genes have been successfully localized to chromosome 8, region 1.1 to 1.8. Homologous segments of this chromosome have been identified in mouse and man.

Publications: 88/01 to 88/12
WOMACK, J.E. Genetic engineering in agriculture: animal genetics and development. Trends in Genetics 3:65-68, 1987.
WOMACK, J.E. A gene map of the cow. Genetic Maps 4:449-501, 1987.
SKOW, L.C., ADKISON, L., WOMACK, J.E., BEAMER, W.G. and TAYLOR, B.A. Mapping of mouse fibronectin gene (Fn-1) to chromosome 1: conservation of the Idh-1-Cryg-Fn-1 synteny group in mammals. Genomics 1:283-286, 1987.
ADKISON, L.R., LEUNG, D.W. and WOMACK, J.E. Somatic cell mapping and restriction fragment analysis of bovine alpha and beta interferon gene familes. Cytogent. and Cell Genet. 47:62-65, 1988.
WOMACK, J.E. Molecular cytogenetics of cattle: a genomic approach to disease resistance and productivity. J. Dairy Sci. 71:1116-1123, 1988.
ADKISON, L.R., SKOW, L.C., THOMAS, T.L., PETRASH, M. and WOMACK, J.E. Somatic cell mapping and restriction fragment analysis of bovine genes for fibronectin and gamma crystallin. Cytogenet. and Cell Genet. 47:155-159, 1988.
HALLERMAN, E.M., THEILMANN, J.L., BECKMAN, J.S., SOLLER, M. and WOMACK, J.E. Mapping of bovine prolactin and rhodopsin genes in hybrid somatic cells. Animal Genet. 19:123-131, 1988.

31.044* CRIS0095764
PEPTIDE AGAINST BOVINE HEAT-STABLE ENTEROTOXIN BY IN-VITRO DIRECTED MUTAGENESIS

SRIRANGANATHAN N; BOYLE S; SCHURIG G; College of Vet Medicine; Va-md Regional Coll of Vet Med, Blacksburg, **VIRGINIA** 24061.
Proj. No.: VA-135185 Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 85 to 30 SEP 88

Objectives: To produce a peptide that is non-toxic but would bind to the heat-stable enterotoxin (STa) receptor i.e., cross reacting material (CRM).

Approach: This will be accomplished by in-vitro directed mutagenesis of STa gene by specific amino acid substitution. Then the mutant STa gene will be ligated into appropriate vector and used in the transformation of E. coli. Using antibodies produced against native STa, we will select transformants (with synthetic gene) that produces STa-like material and test these potential CRM in suckling mouse assay for their ability to block the action of native STa.

Progress: 87/01 to 88/09. A Heat-Stable enterotoxin (STa) positive clone (IX) was created by introducing a Taq I fragment containing STa gene from pSLM004 into the multiple cloning site of M13mp18.Synthetic DNA oligomers representing the proposed amino acid substitutions were used as primers in the enzymatic synthesis of the mutant on the single stranded phage DNA. The heteroduplexes thus generated were utilized in the transformation of Su2 suppressor bearing E. coli JM83 to selectively inhibit the replication of the wildtype STa sequence. Once enriched the mutant STa was moved back into JM109. The specific activities of the two such purified mutant peptides were comparable to the specific activity of native peptide. This suggested that the mutations did not greatly alter the biological activity. The third mutant proposed is being produced on a large scale and will be tested for its biological activity in suckling mice. The mutant DNA sequences so far generated appears to be on the flanking regions of the site of directed mutagenesis. Attempts are under way to extend the DNA sequence generated by altering the conditions of enzymatic synthesis. Amino acid sequence will be

determined of purified mutant peptides to confirm the mutation.

Publications: 87/01 to 88/09
NO PUBLICATIONS REPORTED THIS PERIOD.

31.045* CRIS0135514
CHARACTERIZATION OF THE BRUCELLA ABORTUS GENOME BY PHYSICAL AND GENETIC TECHNIQUES

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Proj. No.: VA-137117Project Type: ANIMAL HEALTH
Agency ID: CSRS Period: 01 OCT 88 to 30 SEP 91

Objectives: Generate a physical map of the B. abortus genome utilizing pulse field gel electrophoresis. Generate a transposon map of the B. abortus genome utilizing P1::Tn5 mutagenesis. Construct a macro-restriction map of B. abortus genome by combining the transposon and the physical maps.

Approach: DNA from avirulent and virulent B. abortus strains will be hydrolyzed with restriction enzymes recognizing rare sequences; the digested genomic fragments will be seperated by pulse field get electrophoresis, strains of B. abortus will be infected with P1::Tn5; the DNA from Kn resistant clones will be characterized by pulse field gel electrophoresis. A macro-restriction map will be constructed by comparing the mobility of the DNA fragments from uninfected cells to those infected with P1::Tn5 on a pulse field gel electrophoreogram.

31.046* CRIS0135245
THE NATURE AND CONSEQUENCES OF SUPEROXIDE DISMUTASE EXPRESSION IN BRUCELLA ABORTUS

BOYLE S M; MISRA H; SCHURIG G; Regional College of Vet Med; Virginia Poly Inst, Blacksburg, **VIRGINIA** 24061.
Proj. No.: VA-135257 Project Type: HATCH
Agency ID: CSRS Period: 15 JUL 88 to 30 JUN 91

Objectives: These experiments will allow the identification, isolation and characterization of the superoxide dismutase (sod) genes of Brucella abortus. An assessment will be made of sod genes structure and function as well as of the relationship of sod gene expression to the ability of Brucella to replicate as an intracellular pathogen.

Approach: The sod genes will be cloned by complementation of sod deficient E. coli or screening by hybridization of a genomic library of Brucella DNA with the sodA and sodB genes of E. coli. The sod genes will be characterized by nucleotide sequencing and expression in E. coli mini-cells. The sod genes will be mutagenized either with the transposon Tn5 or by creation of anti-sense sod genes. The ability of sod deficient Brucella to replicate in mice will be used to measure any changes in virulence.

31.047* CRIS0141611
GENETIC ANALYSIS OF VIRULENT AND AVIRULENT BOVINE HERPESVIRUSES

SHEN D T; KNOWLES D P; GORHAM J R; Agricultural Research Service, Pullman, **WASHINGTON** 99164.
Proj. No.: 5348-34000-002-00D
Project Type: INHOUSE
Agency ID: ARS Period: 05 JAN 87 to 05 JAN 92

Objectives: 1) Establish and compare physical and functional genetic maps of BHV-1 strains; 2) Identify regions of divergence among virulent and modified strains; 3) Clone regions containing diverging nucleotide-sequences; 4) Characterize and express the gene-products encoded in these sequences; 5) Prepare diagnostic reagents such as gene-probes and monoclonal antibodies; 6) Use these reagents to study in vitro and in vivo expression of such genes.

Approach: Molecular analysis of the genome structure and gene expression of bovine herpesviruses will be used to: 1) Establish and correlate physical and functional genetic maps of virulent and avirulent BHV-1 strains; 2) PrepareC-DNA from viral transcripts of the "immediate early" and "early" stages of virus-replication; 3) Identify and clone DNA, C-DNA sequences which seemto correlate with expression of virus-virulence; 4) Produce sensitive nucleic acid probes and immunologic reagents with which such DNA sequences or their gene products can be detected; and 5) Evaluate by in vitro and in vivo tests the diagnosatic practicability of such probes and reagents for the detection of specific DNA sequences of gene products.

Progress: 88/01 to 88/12. Fragments of DNA generated by cleavage of viral DNA of BHV-1 strain LA and BHV-4 strain DN599 with the restriction endonuclease Hind III were ligated into plasmids and amplified by replication in E. coli strain DH 5 a. Purified recombinant DNA was labeled enzymatically with biotin by nick-repair--or random-primed repair synthesis, or photochemically by reaction with photobiotin-acetate. Binding of labeled DNA to immobilized target-DNA was detected with a strepto- vidin-alkaline phosphatase detection system. All three labeling techniques produced probes of comparable specificity for homologous sequences in the target DNA; however, random-primed probes gave the strongest signals. Because of the low cost and simplicity we used the photo-labeling method to compare the ability of the recombinant- DNA probes to detect homologous DNA against a background of bovine DNA. The results indicated that both viral DNAs exhibited unique sequences which can be used to identify viral DNA in nucleic hybridization tests.

Publications: 88/01 to 88/12
GUO, W.Z., SHEN, D.T., EVERMANN, J.F., GORHAM, J.R. 1988. Comparison of an enzyme-linked immunosorbent assay and a complement fixation test for the detection of IgG to bovine herpesvirus type 4 (bovine cytomegalovirus). 9th Annual.

continued - Western Food Animal Disease Research Conference. Moscow, Idaho. (Abstract).
BURGER, D., SHEN, D.T., GORHAM, J.R. 1988. Detection of Bovine Herpesvirus Type 1 (BHV-1) and Bovine Herpesvirus Type 4 (BHV-4) DNA by Hybridization with Non-radioactive Nucleic Acid Probes. 9th Annual Western Food (continued).
(continued) - Animal Disease Research Conference. Moscow, Idaho (abstract).
GUO, W.Z., SHEN, D.T., EVERMANN, J.F., GORHAM, J.R. 1988. Comparison of an enzyme-linked immunosorbent assay and a complement-fixationn test for the strong est signals. Because of the low cost and simplicity we used thephoto-labeling. (5 continued). Am. Journal Vet. Research 49(5) 667-670.

31.048* CRIS0097149
GENETIC ORGANIZATION AND FUNCTION IN THE PRODUCTION OF THE F41 BACTERIAL ADHESIN

MOSELEY S L; Microbiology; University of Washington, Seattle, **WASHINGTON** 98195.
Proj. No.: WNR-8502362 Project Type: CRGO
Agency ID: CRGO Period: 01 SEP 85 to 31 AUG 87

Objectives: Proj 8502362. The proposed research seeks to characterize the genetic basis for the production of E. coli adhesins F41 by enterotoxigenic E. coli. The plasmid or chromosomal location of the gene will be determined, and the number and function of gene products will be analyzed. The genetic relatedness of F41 with other E. coli adhesins will be determined. The nucleotide sequence of the DNA encoding the F41 structural subunit will be determined. A specific hybridization probe for the detection of strains of E. coli which product F41 by colony DNA hybridization will be developed.

Approach: The cloned F41 gene will be physically mapped by restriction analysis. The gene will be localized by analysis of transposon Tn5 insertion mutants. The Tn5 insertion mutants, as well as the wild type cloned gene, will be analyzed for expression of products in an E. coli minicell system. Function of the various products will be determined by phenotypic expression by the insertion mutants of antigen and hemagglutination properties. Genetic relatedness will be studied by Southern blot hybridization of F41-encoding DNA with DNA encoding other adhesins of E. coli. An appropriate restriction fragment hybridizing only to the F41 gene will be developed as an F41 specific hybridization probe.

Progress: 87/01 to 87/08. Genes encoding the production of the F41 bacterial adhesin have been isolated from an enterotoxigenic Escherichia coli strain pathogenic for pigs, and characterized. The determminant was found by Southern blot hybridization to be chromosomal in all F41-producing strains exmined. Four gene products were identified by E. coli maxicell analysis to be associated with F41 production, ad two additional genes were identified by nucleotide sequence analysis. The genes were physically mapped. The entire DNA region encoding F41 was found to share extensive homology with the K88 determinant of E. coli, with the exception of the regions encoding the fimbrial subunits which were non-homologous. The genetic organization of F41 was very similar to that of K88. Nucleotide sequence analysis of the structural subunit gene of F41 predicted a product with structural similarities to K88 and other E. coli fimbriae. K88 and F41 specific hybridization probes were derived from the structural subunit genes of each determinant. Hybridization probes were used to examine a number of E. coli isolates from animals and humans. Some animal isolates reacting with probes derived from sequences shared by K88 and F41 determinants produced neither K88 nor F41. Several of these strains produced fatal septicemia in newborn colostrum-deprived pigs. The probes also detected human enteroinvasive E. coli isolates, and the homology was localized to the large virulence-associated plasmid of these strains.

Publications: 87/01 to 87/08
MOSELEY, S.L., DOUGAN, G., SCHNEIDER, R.A. and MOON, H.W. 1986. Cloning of chromosomal DNA encoding the F41 adhesin of enterotoxigenic Escherichia coli and genetic homology between adhesins F41 and K88. J. Bacteriol. 167:799-804.
RUNNELS, P.L., MOSELEY, S.L., MOON, H.W. 1987. F41 pili as protective antigens of enterotoxigenic Escherichia coli that produce F41, K99, or both pilus antigens. Infect. Immun. 55:555-558.

31.049* CRIS0096211
A POLYVALENT VACCINIA VIRUS RECOMBINANT VACCINE FOR BLUETONGUE

BREEZE R G; GORHAM J R; College of Vet Medicine; Washington State University, Pullman, **WASHINGTON** 99164.
Proj. No.: WNV-2-2608
Project Type: SPECIAL GRANT
Agency ID: CSRS Period: 01 SEP 85 to 31 AUG 87

Objectives: The objective is to make progress toward the development of a polyvalent vaccine for bluetongue through cloning the gene for the serotype specific protein of BTV-13 and then expressing it in an infectious vaccinia virus recombinant.

Approach: The approach includes: Identification of the gene segment which codes for the serotype specific protein which induces protective immunity for BTV-13; cloning of the gene for this serotype specific protein; construction of an infectious vaccinia virus vector which expresses the immunogenic protein of BTV-13; and demonstration of humoral and cellular immunity and protection against live BTV-13 challenge in mice and sheep vaccinated with this vaccinia vector.

Progress: 86/01 to 86/12. We have made monoclonal antibodies to blue tongue virus serotype 13, and these are presently being characterized. We have also isolated the RNAs

of the 10 segments of the virus. Cloning work has not been intiated.

Publications: 86/01 to 86/12
NO PUBLICATIONS REPORTED THIS PERIOD.

31.050 CRIS0027332
METABOLISM AND MECHANISM OF ACTION OF VITAMIN D

DELUCA H F; SCHNOES H K; Biochemistry; University of Wisconsin, Madison, **WISCONSIN** 53706.
Proj. No.: WIS01114 Project Type: STATE
Agency ID: SAES Period: 01 JAN 60 to 30 JUN 90

Objectives: Delineate at the molecular level the metabolism and mechanism of action of vitamins A and D, and to determine hormones and nutritional factors which modulate their activity.

Approach: The primary approach is to synthesize radioactive vitamin A and vitamin D compounds, inject them into vitamin deficient animals, extract their tissues and determine by chromatographic methods the compounds to which they are converted. Once new metabolities are identified, they are isolated in pure form and their structures determined by mass spectrometry and nuclear magnetic resonance spectrometry. The structures of these compounds are then prepared by chemical synthesis and their biological activity determined in animals. Their use in agriculture and medicine are also tested when appropriate. The tissue conversions bringing about these compounds are searched for at the enzymatic or subcellular level; the enzymes are isolated and studied from a physical-chemical point of view. The mechanism of action of the final target hormone in the target tissue is determined by molecular biological techniques.

Progress: 88/01 to 88/12. The full length cDNA encoding for the entire 1,25-dihydroxyvitamin D(3) (1,25-(OH)(2)D(3)) rat receptor has been cloned, and its sequence determined. The deduced amino acid sequence agrees exactly with partial amino acid sequence obtained on pure isolated porcine intestinal 1,25-(OH)(2)D(3) receptor. The latter was obtained by means of an immunoaffinity column in which a monoclonal antibody against the porcine receptor was immobilized on Sepharose CL4B. The structure of the rat receptor is largely homologous to the reported human receptor structure in the DNA binding region and the ligand binding region. Continued attempts to demonstrate the presence of a 1,25-(OH)(2)D(3) responsive element in the calcium binding protein gene isolated from the rat has not met with success. By means of reporter genes we have probed the 5' and 3' ends of the calcium binding protein gene and the introns but none of these regions appear to have a sequence that results in stimulation of transcription of the CAT reporter system. We have developed a new chemical synthesis in which we can rapidly alter the side chain structure of 1,25-(OH)(2)D(3). We have prepared a large number of side chain analogs using this approach. Testing of some of them have revealed that by elongating the side chain in the 24-position, we can construct a compound that is active in causing differentiation but which has no activity in mobilizing calcium.

Publications: 88/01 to 88/12
DARWISH, H.M., KRISINGER, J., STROM, M. and DELUCA, H.F. 1987. Molecular cloning of the cDNA and chromosomal gene for vitamin D-dependent calcium-binding protein of rat intestine. Proc. Natl. Acad. Sci. USA 84:6108-6111.
OSTREM, V.K., LAU, W.F., LEE, S.H., PERLMAN, K., PRAHL, J., SCHNOES, H.K., DELUCA, H.F. and IKEKAWA, N. 1987. Induction of monocytic differentiation of HL-60 cells by 1,25-dihydroxyvitamin D analogs. J. Biol. Chem. 262:14164-14171.
KUTNER, A., PERLMAN, K.L., LAGO, A., SCHNOES, H.K. and DELUCA, H.F. 1988. Novel convergent synthesis of side-chain-modified analogues of 1 alpha,25-dihydroxycholecalciferol and 1 alpha,25-dihydroxyergocalciferol.
DELUCA, H.F. 1988. The vitamin D story: A collaborative effort of basic science and clinical medicine. FASEB J. 2:224-236.
BURMESTER, J.K., MAEDA, N. and DELUCA, H.F. 1988. Isolation and expression of rat 1,25-dihydroxyvitamin D(3) receptor cDNA. Proc. Natl. Acad. Sci. USA 85:1005-1009.
BROWN, T.A., PRAHL, J.M. and DELUCA, H.F. 1988. Partial amino acid sequence of porcine 1,25-dihydroxyvitamin D(3) receptor isolated by immunoaffinity chromatography. Proc. Natl. Acad. Sci. USA 852454-2458.
DELUCA, H.F., SICINSKI, R.R., TANAKA, Y., STERN, P.H. and SMITH, C.M. 1988. Biological activity of 1,25-dihydroxyvitamin D(2) and 24-epi-1,25-dihydroxyvitamin D(2). Am. J. Physiol. 254:E402-E406.

31.051 CRIS0138341
WITHIN FAMILY LINKAGE BETWEEN RFLP MARKERS AND ECONOMICALLY IMPORTANT TRAITS OF DAIRY CATTLE

DENTINE M R; Dairy Science; University of Wisconsin, Madison, **WISCONSIN** 53706.
Proj. No.: WIS03303 Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 89 to 30 SEP 93

Objectives: To investigate the linkage of a set of restriction enzyme fragment length polymorphisms as marker alleles for quantitative traits in dairy cattle and to estimate genetic parameters of the marker alleles.

Approach: Laboratory procedures to detect restriction fragment length polymorphisms will be extended for use on DNA from somatic cells in milk. Milk samples and records on yields of daughters of sires heterozygous for marker alleles will be collected from Wisconsin herds. Cows will be characterized on the basis of genotype for marker alleles associated with the bovine prolactin gene. A statistical model incorporating a mixture of major genes and underlying quantitative variation will be employed. Data will be used to obtain conditional Maximum Likelihood estimates of the

linkage effects, gene frequencies and recombination losses. Gene frequencies in the population will be estimated using samples of unrelated herdmates of these cows.

31.052 CRIS0091397
GENETIC POLYMORPHISMS IN GENES CODING FOR PROLACTIN, GROWTH HORMONE AND PLACENTAL LACTOGEN IN COWS

SCHULER L A; School of Veterinary Medicine; University of Wisconsin, Madison, **WISCONSIN** 53706.
Proj. No.: WIS02819 Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 83 to 30 SEP 86

Objectives: To determine the extent and location of polymorphisms in the genes coding for the lactogenic hormones, prolactin, growth hormone and placental lactogen in cows, and correlate these findings with breed or production traits.

Approach: DNA prepared from semen of representative dairy, beef and exotic breeds will be examined for polymorphic loci by digesting the genomic DNA with various restriction enzymes, separating the fragments on an agarose gel, transferring the DNA to nitrocellulose and hybridizing to nick-translated cDNA to examine the coding regions, and unique sequences from the flanking regions of these genes (Southern hybridizations). Areas of interest will be sequenced after cloning into M13 vectors by the method of Sanger. If positive results are indicated by the above studies, larger studies will be set up as appropriate depending on the findings above to correlate breed and use. DNA from potential families will be examined as available.

Progress: 83/10 to 85/09. In order to study polymorphisms in the bovine growth hormone gene family, the postulated third member (in addition to prolactin and growth hormone), placental lactogen, needed to be characterized at the gene level. This area was most fruitful: We isolated and sequenced a cDNA from a cDNA library made from mRNA from fetal cotyledonary tissue. This cDNA displayed about 70% homology to bovine prolactin, and little similarity to bovine growth hormone. This suggests a different evolutionary origin for the placental hormone in this species, as opposed to the primate hormone. This cDNA was about 1100 bp in length, consistent with the increased size of the isolated protein. A second type of cDNA, also homologous to prolactin, was isolated. This was as different from the first, as the first was from prolactin. We concluded that the bovine growth hormone gene family was much more complicated than originally thought. Genomic Southern blots showed a complex pattern indicating multiple genes, with regions coding for the amino terminals displaying more homology. Analysis of the extent of polymorphic regions in all genes in DNA prepared from both blood and semen from 14 bulls representing 6 breeds showed considerable variation in gene structure. These variations appeared to be individual variation, and did not correlate closely with either use or breed.

Publications: 83/10 to 85/09
SCHULER, L.A., HURLEY, W.L. and GORSKI, J. The gene for placental lactogen in the cow appears to have evolved from prolactin rather than growth hormone. 7th International Congress of Endocrinology, Quebec City, 1984. Abs. 2138.
SCHULER, L.A. and HURLEY, W.L. Evolution of bovine placental lactogen includes insertion 5′ to the second exon. Endocrine Society, 67th Annual Meeting, Baltimore, 1985, Abs. 528.

31.053 CRIS0136050
CHARACTERIZATION OF BOVINE MHC CLASS I AND II GENES IN THE LYMPHOBLASTOID CELL LINE BL3

SPLITTER G; Veterinary Science; University of Wisconsin, Madison, **WISCONSIN** 53706.
Proj. No.: WIS03241 Project Type: CRGO
Agency ID: CRGO Period: 15 JUL 88 to 31 JUL 90

Objectives: PROJ. 8800353. Using DNA sequencing, isolate by DNA cloning and characterize MHC class II genes.

Approach: Prepare genomic and cDNA libraries of bovine DNA. Isolate clones from the library containing fragments hybridizing with equivalent human DNA probes. Determine unique restriction sites and sequence the cloned fragments. Compare the sequenced fragments with the human and murine sequences.

31.054 0141672
CULICOIDES VARIIPENNIS; VECTOR BIOLOGY AND VECTOR COMPETENCE FOR BLUETONGUE VIRUS

WALTON T E; NUNAMAKER R A; HOLBROOK F R; Agricultural Research Service, Laramie, **WYOMING** 82070.
Proj. No.: 5410-34350-001-00D
Project Type: INHOUSE
Agency ID: ARS Period: 01 NOV 86 to 01 NOV 91

Objectives: Investigate variability and systematic status of C. variipennis (CV). Improve procedures for colonization and maintenance of biting midges. Investigate vector-virus-host interactions in BTV cycle. Improve sampling techniques for arthropod vectors. Evaluate seasonal dynamics of the vector-BTV relationship and produce a predictive model. Characterize genetics of CV competence for BTV.

Approach: Electrophoretic, chromatographic (isozyme/hydrocarbon) and EM studies of CVpopulations will be correlated to BTV vector potential. Specific survey techniques will be utilized to evaluate vector-BTV events, correlate with meteorological events and develop a BTV predictive model. Evaluations of vector-virus survey techniques will continue in

Wyoming, Colorado and Nebraska. Biologically secure rearing and handling systems for genetically defined CV colonies will be evaluated and a standard manual of techniques prepared. Selected genetic lines of CV will be assayed for oral suscepti- bility to BTV and phenotypic analyses developed using traditional and molecular biologic techniques. Selected lines will be genotypically analyzed for vector competence, biochemical characteristics and morphological characters. Environmental influences on genetic characteris- tics will be lab evaluated.

Progress: 88/01 to 88/12. Cv is the primary US vector of BTV. Wild & colonized adults were marked with RbCl to study flight range. Two natural, genetically distinct US Cv populations were identified isozymically: Cv variipennis in the northeast & Cv sonorensis in the west. Natural Cvs populations show seasonal genetic changes. Established isofemale Cvs lines of known family groups to characterize by infection rates & competence. Cloned Cvs DNA fragments to study genetic diversity. High incubation temperature (26C vs 20C) increased Cvs mortality. Infection with 3 different BTV serotypes caused no differences in Cvs mortality. Examined adult Cv midguts using immunogold-labelled ultrathin cryosections (IGLUC) after infection with BTV-11; BTV was seen on/within erythrocytes & in midgut cells. IGLUC with monoclonal antibody detected BTV in Cv developing oocytes: BTV was not transmitted transovarially to offspring thru 5 gonatrophic cycles, but antigen was detected in yolk bodies & vitelline membrane of oocytes indicating BTV may penetrate the ovarian sheath. Each of the paired Cv salivary glands consists of 5 lobes attached at the gland's base. Glandular cells had abundant endoplasmic reticulum, mitochondria & microtubules; a basal lamina borders the epithelial cells lining the salivary duct. IGLUC detected BTV antigen in cytoplasm & plasma membrane of salivary gland acinar cells & detected mature virions & antigen extracellularly & in cisternae of vacuoles & endoplasmic reticulum.

Publications: 88/01 to 88/12

NUNAMAKER, R.A., WICK, B.C. and NUNAMAKER, C.E. 1988. Salivary glands of female Culicoides variipennis (Diptera:Ceratopogonidae): Morphologic changes associated with maturation and blood-feeding. Proc. International Congr. Entomol. 28:90.

NUNAMAKER, R.A., WICK, B.C. and NUNAMAKER, C.E. 1988. Immunogold labelling of bluetongue virus in cryosections from Culicoides variipennis (Coquillett) salivary gland. Proc. Electron Microscopy Soc. Amer., 372-373.

FRANCIS, B.R., BLANTON, W.E., LITTLEFIELD, J.L. and NUNAMAKER, R.A., Hydrocarbons of the cuticle and hemolymph of the adult honey bee (Apis mellifera Linnaeus). Annals Entom. Soc. Am., Accepted October 25, 1988.

SIEBURTH, P.J. and MARUNIAK, J.E. 1988. Growth characteristics of a cell line from the velvetbean caterpillar, Anticarsia gemmatalis Hubner (Lepidoptera: Noctuidae). In Vitro Cell Devel. Biol. 24:195-198.

SIEBURTH, P.J. and MARUNIAK, J.E. 1988. Susceptibility of a cell line of Anticarsia gemmatalis (Lepidoptera:Noctuidae) to three nuclear polyhedrosis viruses. J. Inverteb. Pathol. 52:453-458.

HOLBROOK, F.R. 1988. Bluetongue in the United States: Status, transmission and control through vector suppression. Bull. Soc. Vector Ecol. 13:350-353.

AKEY, D.H., LUEDKE, A.J. and OSBURN, B.I. 1988. Development of hypersensitivity in cattle to the biting midge (Diptera:Ceratopogonidae). Misc. Publ. Ent. Soc. Amer. 71:22-28.

32.001* CRIS0136409
SEX-SPECIFIC DNA IN LIVESTOCK ANIMALS

MCGRAW R A; College of Vet Medicine; University of Georgia, Athens, **GEORGIA** 30602.
Proj. No.: GEOV-0186 Project Type: STATE
Agency ID: CSVM Period: 01 JUL 87 to 30 JUN 91

Objectives: This project is aimed at identifying and characterizing sex-specific DNAs in economically important livestock species. The basic genetic information can be used to develop sex-specific DNA probes with potential application in assays for sex-fractionation of semen and/or sex determination of embryos.

Approach: The approach is to compare DNAs derived from male and female animals of each species by a variety of molecular genetic methods, including restriction analysis, cloning, sequencing, and hybridization techniques. DNA sequences unique to one of the sexes are then characterized and developed as sex-specific hybridization probes.

Progress: 87/07 to 88/12. This research is aimed at identifying and characterizing sex-specific DNAs in economically important livestock species. The genetic information is used to develop sex-specific DNA probes with potential application in assays for sex-fractionation of semen and in sex-identification of embryos. Methods include a variety of DNA manipulations: restriction enzyme digestions, electrophoretic separations, construction and propagation of recombinant DNA in bacteria, DNA sequece analysis, chemical DNA synthesis, enzymatic DNA amplification, and hybridizations using radioactively labelled probes. At this time, we have developed sex-specific probes in pigs and chickens. The procine probe has been used successfully for sex-identification of procine embryos and efforts are underway to attempt sex-fractionation of boar semen. Preliminary data suggests that we will be able to develop similar probes in horses and cattle.

Publications: 87/07 to 88/12
MCGRAW, R.A., JACOBSON, R.J. and AKAMATSU, M. 1988. A male-specific repeated DNA sequence in the domestic pig. Nucleic Acids Research 16(21):10389.

32.002 CRIS0133947
THE SWINE HISTOCOMPATIBILITY COMPLEX AND ITS ASSOCIATION WITH HEALTH AND PRODUCTION

SCHOOK L B; Animal Science; 1301 West Gregory Drive, Urbana, **ILLINOIS** 61801.
Proj. No.: ILLU-35-0328 Project Type: HATCH
Agency ID: CSRS Period: 15 JAN 88 to 30 SEP 92

Objectives: To develop a linkage map of the class II region of the SLA using families of Landrace or Duroc pigs. To perform restriction fragment length polymorphism (RFLP) analysis of MHC class I, class II, and class III genes within families as a means of characterizing and studying the segregation of haplotypes. To establish associations between SLA haplotypes and disease susceptibility, reproductive performance, and meat production.

Approach: It is the aim of this study to examine the MHC of pigs, commonly called the SLA because it encodes polymorphic swine leukocyte antigens. Immunological and molecular techniques will be used to construct a linkage map of the SLA and to assign distinctive DNA restriction patterns to known haplotypes. Characterization of these genes will then permit studies on the association between SLA alleles and haplotypes with production and disease resistance/susceptibility. This will lead to future studies in which we will clone and characterize these genes for use in gene transfer experiments to further analyze the relationship between SLA alleles and their function.

Progress: 87/10 to 88/09. A panel of serological reagents that recognizes putative allelic products of the swine major histocompatibility complex (SLA) are being developed. Over 500 primiparous sera have been screened against Landrace, Duroc, Yorkshire, Hampshire and three inbred lines of NIH miniature pigs. Cluster analysis was performed and clusters of sera were r (>-).85 were further studied. Two clusters which correlated with the RBC A system were identified and designated as A and A.1. A.1 was included in A. Because of the anti-A activity in a large number of the sera screened and the apparent complexity of the non-A antibodies in parous sera, clusters were generated independently within each breed and then only those clusters that reacted with three or more animals were selected. Eighteen distinct lymphocyte alloantigens (non-A) were identified. Each specificity is being further studied by segregation analysis in full sib families and correlation with reference reagents provided by S. Lazary and C. Gautschi, Berne, Switzerland. Additional work has focused on using RFLP analysis to define distinctive DNA restriction patterns for SLA class I and class II genes. This approach is being used to define SLA homozygotes for use as typing cells and in studies aimed at determining the relationship between SLA and health and production traits.

Publications: 87/10 to 88/09
SHIA, Y.-C., WU, M.-C., LEWIN, H.A. and .SCHOOK, L.B. (1987). Allogenotopes of MHC class I and class II genes in pigs. Midwest Immunol. Conf. 16:85.
WU, M.-C. SHIA, Y.-C., McLAREN, D.G., LEWIN, H.A. and SCHOOK, L.B. (1988). Ten serologically-defined lymphocyte alloantigens identified in North American pigs. In: The Molecular Biology of the Major Histocompatibility Complex of Domestic A.
SHIA,Y.-C., WU, M.-C., McLAREN, D.G., LEWIN, H.A. and SCHOOK, L. B. (1989).
Swine MHC class I and class II RFLP. Anim. Gen., In press.
WU, M.-C., SHIA, Y.-C., LING, M.S., WANG, T.L., STEWART, J.A., BEEVER, J.E., McLAREN, D.G., LEWIN, H.A. and SCHOOK, L.B. (1989). Serologically-defined lymphocyte alloantigens in North American pigs. Anim. Gen., In Press.

32.003* CRIS0077856
IMMUNOLOGY AND PATHOGENESIS OF PARASITIC DISEASES OF ANIMALS

KAZACOS K R; Veterinary Pathobiology; Purdue University, West Lafayette, **INDIANA** 47907.
Proj. No.: IND073029 Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 88 to 30 SEP 93

Objectives: For selected naturally occurring parasitic diseases of animals, determine: Immune responses, mechanisms, and immunodiagnosis. Pathogenesis, pathology, and parasitologic parameters. Prevalence and epidemiologic patterns. Focus these studies on the following diseases, and others as they might arise: Ascariasis (Ascaris suum) of swine. Trichinellosis (Trichinella spiralis of swine and wildlife. Nematode larva migrans diseases of animals (Baylisascaris, Tococara).

Approach: Naturally occurring and experimentally induced cases of selected parasitic diseases will be studied by laboratory and field observatios and methods, including the application of modern immunochemical and molecular biologic techniques.

Progress: 87/10 to 88/09. Twenty-one wildlife isolates of Trichinella spiralis were analyzed using DNA restriction fragment length polymorphisms (RFLPs). Similar RFLP patterns were seen with enzymes Eco RI, Hae III, Hpa II, Hind III and Xba I. Cla I digestion produced unique RFLPs indicating that the sylvatic group is a heterogeneous complex. Swine Trichinella RFLP was different from sylvatic Trichinella RFLPs. RFLP analysis and Southern blot hybridizations using a U.S.D.A. swine Trichinella-specific DNA probe confirmed that one coyote isolate actually represented sylvatically-maintained swine Trichinella. This has important implications for the epidemiology of swine trichinosis. Research on the immunodiagnosis of Baylisascaris larva migrans indicated the close relationship between B. melis and B. procyonis, based on gradient SDS-PAGE of larval excretory-secretory (ES) proteins. B. columnaris ES was less like the other two, and B. transfuga ES was most dissimilar. Carbohydrate staining and biotinylated lectin binding on Western blots indicated that B. procyonis ES antigens were complex glycoproteins with many sugars represented. Immunologic cross-reactivity was directed primarily at carbohydrate epitopes. Work was initiated to produce and characterize monoclonal antibodies against B. procyonis ES. Other research indicated that a portable flame gun is potentially useful for decreasing transmission of the swine roundworm Ascaris suum in growing-finishing swine units, through destruction of the resistant eggs.

Publications: 87/10 to 88/09

BOYCE, W.M., KAZACOS, E.O., KAZACOS, K.R. and ENGELHARDT, J.A. (1987). Pathology of pentastomid infections (Sebekia mississippiensis) in fish. J. Wildl. Dis. 23:689-692.

BOYCE, W.M., BRANSTETTER, B.A. and KAZACOS, K.R. (1988). In vitro culture of Baylisascaris procyonis and initial analysis of larval excretory-secretory antigens. Proc. Helminthol. Soc. Wash. 55:15-18.

KAZACOS, K.R. and KAZACOS, E.A. (1988). Diagnostic exercise: Neuromuscular condition in rabbits. Lab. Anim. Sci. 38:187-189.

BOYCE, W.M., BRANSTETTER, B.A., and KAZACOS, K.R. (1988). Comparative analysis ot larval excretory-secretory antigens of Baylisascaris procyonis, Toxocara canis and Ascaris suum by western blotting and enzyme immunoassay.

DIXON, D., REINHARD, G.R., KAZACOS, K.R., and ARRIAGA, C. (1988). Cerebrospinal nematodiasis in prairie dogs from a research facility. J. Am. Vet. Med. Assoc. 193:251-253.

HAMANN, K.J., KEPHART, G.M., KAZACOS, K.R., and GLEICH, G.J. Immunofluorescent localization of eosinophil granule major basic protein in fatal human cases of Baylisascaris procyonis infection. Am. J. Trop. Med. Hyg,. In press.

LITTLE, A.S. (1987). Immunological comparison of larval Baylisascaris procyonis, Toxocara canis, and Ascaris suum using immunodiffusion. M.S. Thesis, Purdue University, West Lafayette. 101 p.

32.004* CRIS0131805
FACTORS INFLUENCING EMBRYO SURVIVAL IN DOMESTIC FARM ANIMAL SPECIES

FORD S P; Animal Science; Iowa State University, Ames, **IOWA** 50011.
Proj. No.: IOW02825 Project Type: HATCH
Agency ID: CSRS Period: 01 JUL 87 to 30 SEP 91

Objectives: Investigate the effect of SLA haplotype on the rate of development and survival of pig embryos, and develop plans to improve litter size by identifying SLA haplotypes of breeding pairs. Continue investigations on hormonal control of uterine blood flow throughout pregnancy in the ewe, cow and sow, with emphasis on the role of the uterine vasculature in conceptus growth and survival. In vitro perfusion of the bovine placentome as a "mini placenta" to study the factors which control the flow of blood to the fetal-maternal interface in the cow as well as factors which control bovine placental steroid production.

Approach: Identify SLA haplotypes in pigs by restriction fragment length polymorphism analysis. Relate these SLA haplotypes to developmental rate of embryos by: identification of SLA antigens on very early pig embryos utilizing an enzyme-linked immunosorbent assay (ELISA), counting the number of blastomeres of preimplantation pig embryos of different SLA haplotypes during defined periods during early pregnancy, and evaluation of the relative survival of embryos of different SLA haplotypes. Use chronic in vivo measurement of uterine blood flow (electromagnetic flow probes, microspheres, etc.), and in vitro perfusion of the bovine placentome, to investigate factors which control flow through the uterine and placental

vasculatures throughout the periods of embryo and fetal development.

Progress: 88/01 to 88/12. Effect of PGF(subscript 2)(Alpha) on porcine corpora lutea (CL) following administration on day 9 of the estrous cycle. During the period prior to day 12 of the estrous cycle, porcine CL refractory to the luteolytic effects of PGF(subscript 2)(Alpha). We investigated functional and structural aspects of the effects of PGF(subscript 2)(Alpha) on porcine CL during the refractory period. Gilts were unilaterally ovariectomized on day 8 and utero-ovarian venous (UOV) and femoral arterial (FA) catheters were inserted. Gilts received 20 mg PGF(subscript 2)(Alpha) or vehicle on day 9 and the remaining ovary was removed on day 12. Progesterone declined markedly in the FA (3 hrs) and UOV (2 hrs) following PGF(subscript 2)(Alpha), but not vehicle, and had returned to pretreatment levels by day 11. Luteal growth (weight, protein and DNA content) continued in pigs from day 8 to day 12 and was not affected by a luteolytic dose of PGF(subscript 2)(Alpha) on day 9. These data suggest that PGF(subscript 2)(Alpha) administration on day 9 of the estrous cycle in pigs has transient inhibitory effects on luteal function without effects on luteal composition. Effect of intraluteal estradiol-17(Beta) implants on weight and progesterone secretion of porcine corpora lutea (CL). Estradiol-17(Beta) (E(subscript 2)) decreases the effectiveness of prostaglandin F(subscript 2)(Alpha) (PGF(subscript 2)(Alpha)) to induce luteolysis, and E(subscript 2) locally increases CL wt in pigs suggesting a direct luteotropic effect.

Publications: 88/01 to 88/12

GUENTHER, A.E., CONLEY, A.J., VAN ORDEN, D.E., FARLEY, D.B. and FORD, S.P. (1988). Changing structural and mechanical properties of uterine arteries during porcine gestation. J. Anim. Sci. 66:3144-3152.

FORD, S.P., SCHWARTZ, N.K., ROTHSCHILD, M.F., CONLEY, A.J. and WARNER, C.M. (1988). Influence of SLA haplotype on preimplantation embryonic cell number in miniature pigs. J. Reprod. Fert. 84:99-104.

LUND, J., FAUCHER, D.J., FORD, S.P., PORTER, J.C., WATERMAN, M.R. and MASON, J.I. (1988). Developmental expression of bovine adrenocortical steroid hydrolases: regulation of P-450(subscript 17)(Alpha) expression leads to episodic fetal cor.

CONLEY, A.J. and FORD, S.P. (1989). Effects of a phorbol ester (TPA), calcium ionophore (A23187) and prostaglandin F(subscript 2)(Alpha) (PGF(subscript 2)(Alpha)) on progesterone secretion by dispersed ovine luteal cells. Biol. Reprod.

CONLEY, A.J. and FORD, S.P. (1989). Direct luteotropic effect of oestradiol-17(Beta) on porcine corpora lutea. J. Reprod. Fert. (In press.).

CONLEY, A.J., PUSATERI, A.E. and FORD, S.P. (1989). Effects of prostaglandin F(subscript 2)(Alpha), (PGF(subscript 2)(Alpha)) on porcine corpora lutea (CL) following administration on day 9 or the estrous cycle. Proceedings Midwestern Sect.

32.005 CRIS0089577

RELATIONSHIP BETWEEN THE SLA GENE COMPLEX AND RESISTANCE TO PSEUDORABIES

ROTHSCHILD M F; WARNER C M; CHRISTIAN L L;
Animal Science; Iowa State University, Ames, **IOWA** 50011.

Proj. No.: IOW02609 Project Type: ANIMAL HEALTH
Agency ID: CSRS Period: 22 FEB 83 to 30 SEP 86

Objectives: Continue to determine genetic differences in SLA gene complex of breeds of swinein the U.S. using newly developed typing reagents as they become available. Examine differences in immune response of pigs to immunization with a modified live pseudorabies virus vaccine (MLPRV). Correlate the relationship between SLA haplotypes and immune response to MLPRV.

Approach: Immunize baby pigs at 4 weeks of age. Bleed pigs at 8 weeks of age and then measure antibody titer using a serum neutralization method. PRV antibodies levels will then be correlated to SLA haplotypes which are determined using microcytotoxicity tests and using conventional antisera. Monoclonal antibodies will be used as they are developed.

Progress: 83/02 to 86/09. The objectives were to determine genetic differences in the Swine Lymphocyte Antigen (SLA) gene complex and to examine differences in immune response of pigs following vaccination with a modified live pseudorabies virus vaccine (PRV). Genetic differences at the SLA complex were investigated using standard serological reagents obtained from NIH, monoclonal antibodies produced in our laboratory and use of restriction fragment length polymorphism analysis. Results clearly indicate that enormous genetic variability at the SLA gene complex exists which could be exploited. Genetic control of immune response to PRV vaccine also existed as demonstrated in 2 major experiments involving over 2000 pigs of the Chester White, Duroc, Hampshire, Landrace and Yorkshire breeds. Large breed and sire differences for immune response existed but no relationship to the SLA complex was found. The heritability estimate for immune response at 56 days was .18 & the maternal environment accounted for 11% of the phenotypic variance in immune response. No heterosis for immune response was observed. Results indicated that immune response to PRV was antagonistically associated with growth rate and had no relationship to backfat. Results indicate that if high immune response to PRV is desired, selection may be effective. Genetic variability at the SLA complex and results from associated projects indicates that selection based on SLA genotypes may become of practical use to improve health and reproduction in the pig.

Publications: 83/02 to 86/09

WARNER, C.M., MEEKER, D.L. and ROTHSCHILD, M.F. 1987. Genetic control of immune responsiveness: A review of its use as a tool for selection for disease resistance. J. Anim. Sci. (in press).

MEEKER, D.L., ROTHSCHILD, M.F., CHRISTIAN, L.L., WARNER, C.M. and HILL, H.T. 1987. Genetic control of immune response to

pseudorabies and atrophic rhinitis vaccines. I. Heterosis.
MEEKER, D.L., ROTHSCHILD, M.F., CHRISTIAN, L.L., WARNER, C.M. and HILL, H.T. 1987. Genetic control of immune response to pseudorabies and atrphic rhinitis vaccines. II. Comparison of additive direct and maternal genetic effects.
FLANAGAN, M.P., ROTHSCHILD, M.F., SCHWARTZ and WARNER, C.M. 1987. Restriction-fragment length polymorphism analysis of SLA class I genes within the Duroc breed. Proc. 20th Annu. Midwestern Sect. Amer. Soc. Anim. Sci meetings. (submitted).
FLANAGAN, M.P., ROTHSCHILD, M.F., SCHWARTZ and WARNER, C.M. 1987. Inheritance of SLA class I genes in the pig: Determination of class I genotypes by restriction fragment length polymorphism analysis. Proc. FASEB meetings. (Submitted).

32.006 CRIS0096980
ROLE OF THE SLA COMPLEX IN PIG DEVELOPMENT AND REPRODUCTION

WARNER C M; ROTHSCHILD M F; FORD S P; Biochemistry & Biophysics; Iowa State University, Ames, **IOWA** 50011.
Proj. No.: IOW-8502672 Project Type: CRGO
Agency ID: CRGO Period: 15 SEP 85 to 30 SEP 88

Objectives: Proj. 8502672. There are two main objectives of this research: (1) Identification of SLA antigens on very early pig embryos; (2) Identification of a putative SLA-linked Ped (preimplantation embryo development) gene.

Approach: SLA antigens on early pig embryos will be identified by using an enzyme linked immunosorbent assay (ELISA) method. Both conventional antisera and monoclonal antibodies will be used for the assay. The use of dot-blots and Northern blots to detect mRNA in pig embryos will also be attempted. The Ped gene will be searched for in embryos from miniature pigs of known SLA haplotypes. This will require counting the number of cells per embryo at certain times post-mating and correlating rate of development with SLA haplotype.

Progress: 87/10 to 88/09. In the last year of this grant we finished many of our experiments on the genes of the swine major histocompability complex (MHC). We characterized a panel of monoclonal antibodies to swine MCH antigens. We also characterized, by restriction fragment length polymorphism (RFLP) analysis, the MHC genes of Duroc and Hampshire swine. Finally, we continued our studies on embryos from NIH miniature swine of defined MHC haplotypes. The long range goal is to increase reproductive efficiency in swine by determining exactly which MHC-linked genes are involved in the control of reproduction. These genes will be excellent candidates for the future genetic engineering of swine.

Publications: 87/10 to 88/09
CONLEY, A.J., JUNG, Y.C., SCHWARTZ, N.K., WARNER, C.M., ROTHSCHILD, M.F. and FORD, S.P. 1988. Influence of SLA haplotype on ovulation rate and litter size in miniature swine. J. Reprod. Fert., 82, 595-601.
FLANAGAN, M.P., JUNG, Y.C., ROTHSCHILD, M.F. and WARNER, C.M. 1988. RFLP analysis of SLA clAss I genotypes in Duroc swine. Immunogenetics, 27, 465-469.
FORD, S.P., SCHWARTZ, N.K., ROTHSCHILD, M.F., CONLEY, A.J. and WARNER, C.M. 1988. Influence of SLA haplotype on preimplantation embryonic growth rate in miniature swine. J. Reprod. Fert. 84, 99-104.
LIE,W.-R., ROTHSCHILD, M.F. and WARNER, C.M. 1988. Preparation and characterization of murine monoclonal antibodies to swine lymphocyte antigens.
Immunology, 64, 599-605.
JUNG, Y.C., ROTHSCHILD, M.F., FLANAGAN, M.P., CHRISTIAN, L.L. and WARNER, C.M. 1989.
JUNG, Y.C., ROTHSCHILD, M.F., FLANGAN, M.P., POLLAK, E. and WARNER, C.M. 1989. Genetic variability between two breeds based on restriction fragment length polymorphisms (RFLPs) of major histocompatibility complex class I genes in the pig.

32.007 CRIS0131787
MYCOPLASMA HYOPENEUMONIAE RECOMBINANT VACCINE

MINION F C; Veterinary Medical Research Institute; Iowa State University, Ames, **IOWA** 50011.
Proj. No.: IOWV-410-23-53
Project Type: ANIMAL HEALTH
Agency ID: CSRS Period: 01 FEB 87 to 30 SEP 90

Objectives: To develop a recombinant vaccine that will prevent M. hyopneumoniae colonization and disease in swine.

Approach: In order to develop an effective recombinant vaccine against M. Hyopneumoniae we will identify mycoplasmal gene sequences that code for specific surface antigens, clone them onto an expression vector, transform different Salmonella strains with these recombinant plasmids, and evaluate the transformants for vaccine potential in mice and swine.

Progress: 87/01 to 88/09. Most of the efforts the past year have been towards developing better culture methods for M. hyopneumoniae. Current methods in determining colony forming units are unacceptable for genetic work. Better growth and colony forming characteristics are necessary for identifying transposon and interposon mutants. A new media shows promise, but is still under development. In addition we are passing several strains in the laboratory in hopes of improving growth and colony forming characteristics. A lambda gene library was constructed and screened with hyperimmune rabbit and convalescent swine sera. Antigen production in this library is poor, probably as a result of inappropriate gene regulatory signals and prevalence of UGA stop codon within coding sequences. Work is underway to minimize these effects and thereby increase the antigen producing capabilities of this library. Future studies will concentrate on growth characteristics and antigen expression in gene libraries.

Publications: 87/01 to 88/09
No publications reported this period.

32.008 CRIS0134394
PHYSICAL MAPPING MYCOPLASMAL CHROMOSOMES

MINION F C; Veterinary Medical Research Institute; Iowa State University, Ames, **IOWA** 50011.
Proj. No.: IOWV-410-23-83
Project Type: ANIMAL HEALTH
Agency ID: CSRS Period: 01 MAR 88 to 30 SEP 92

Objectives: The major objective of this proposal is to construct a restriction enzyme map of the chromosome of Mycoplasma hyopneumoniae. Several different enzyme sites will be mapped so that future studies using clone banks or other DNA or genetic techniques can benefit.

Approach: A lambda clone bank of M. hyopaeumoniae chromosomal DNA has been constructed in E. coli. From this bank, enough individual plaques will be picked and stored in microtiter plates to ensure a complete representation of the chromosome. Overlapping inserts will be determined using end specific probes generated with T7 and T3 RNA polymerase. This will order the fragments into an overlapping map. Individual clones will then be analyzed by restriction digests using an incomplete digestion and an end specific probe to order restriction sites within the fragment. This data will be analyzed at different stages on a Macintosh II computer system. Eventually a restriction enzyme map of the chromosome will be generated.

Progress: 87/01 to 88/09. Progress has been made in preliminary studies to map mycoplasmal chromosomes using Field Inversion Gel Electrophoresis. Experiments are being performed to establish the system to maximize fragment separation, and also to determine the best molecular weight markers to use. Commercially available lambda molecular weight ladders have proven unsuitable, and therefore we are working to produce our own. A modified cosmid vector is being constructed which will facilitate the mapping considerably. This cosmid will contain flanking NotI restriction enzyme sites, a multiple cloning site, and T7 and T3 RNA polymerase promotor sites. Other unique features of this system include an in vivo packaging strain of E. coli which will facilitate the isolation of sufficient quantities of DNA for restriction analysis and other manipulations. Techniques have also been developed to blot and analyze DNA gels rapidly. This will increase the speed with which the mapping will proceed.

Publications: 87/01 to 88/09
No publications reported this period.

32.009 CRIS0137514
DEVELOPMENT OF A TREPONEMA HYODYSENTERIAE VACCINE

MINION F C; WANNEMUEHLER M; Veterinary Medical Research Institute; Iowa State University, Ames, **IOWA** 50011.
Proj. No.: IOWV-400-63-51 Project Type: STATE
Agency ID: CSVM Period: 01 AUG 88 to 31 JUL 91

Objectives: The genes coding for the Treponema hyodysenteriae LPS O antigen will be cloned in E. coli and used to develop a recombinant vaccine.

Approach: Unique lambda and cosmid libraries will be screened with monoclonal antibodies against T. hyodysenteriae O antigen to identify positive reacting clones. Unique hosts and expression vectors will be used because of the need for expression of enzymatically active gene products and the construction of the O antigen side chain. The genes will be mapped on the vector insert and subcloned into a Salmonella expression vector. The vaccine will be delivered orally using a live recombinant organism and the serum antibody responses monitored. Alternatively, a submut vaccine could be produced from the recombinant E. coli. Protection will be assessed using virulent challenge experiments.

32.010 CRIS0048094
REPRODUCTIVE DISEASE OF SWINE

MENGELING W L; VORWALD A C; Agricultural Research Service; National Animal Disease Ctr, Ames, **IOWA** 50010.
Proj. No.: 3630-34000-022-00D
Project Type: INHOUSE
Agency ID: ARS Period: 28 JUN 83 to 21 JUN 88

Objectives: Determine the epizootiology and pathogenesis of reproductive disease of swine, particularly those caused by viruses, to study the defense mechanisms of the reproductive tract and to formulate methods of reproductive disease control..

Approach: Incidence of infection, modes of dissemination, pathogenesis and mechanisms of immunity to viruses asociated with the reproductive disease of swine will be determined as completely as possible. Research emphasis will be placed on porcine parvovirus and pseudorabies virus which have been shown previously to be the major causes of virus-induced reproductive disease of swine in the U.S. Studies will include identification of immunogenic viral proteins, mapping of viral genomes and development of subunit viral vaccines, and study of defense mechanisms to viral infections in the reproductive tract..

Progress: 87/01 to 87/12. Most of the autonomous parvoviruses infect and cause disease in a single host species. For example, porcine parvovirus (PPV) is ubiquitous among swine and is a major cause of maternal reproductive failure; however, there is no

evidence that it causes disease or even infects any other kind of animal. To better understand the basis for species specificity we investigated the stage of the infectious cycle at which viral replication is restricted in nonpermissive cells. The model system selected for study was PPV and its interaction with porcine kidney (permissive) and bovine spleen (nonpermissive) cells. It was found that the virus was adsorbed to, and entered the cytoplasm of, both types of cells, thus excluding the possibility that only porcine cells have the necessary viral receptors. Moreover, when cells were transfected with "naked" viral deoxyribonucleic acid (DNA) only porcine kidney cells supported viral replication. This observation suggests that intracellular viral uncoating is not a limiting factor and certainly not the only factor in restricted replication. The most likely reason for species specificity, therefore, appears to be the level of transcription of viral DNA. Possible species differences in ribonucleic acid polymerases and associated factors have yet to be investigated.

Publications: 87/01 to 87/12

RIDPATH, J.F., PAUL, P.S., and MENGELING, W.L. 1987. Comparison of porcine parvovirus to other parvoviruses by restriction site mapping and hybridization analysis of southern blots. J. Gen. Virol. 68:895-900.

32.011* CRIS0049916

CLONING DNA OF COCCIDIAN AND HELMINTH PARASITES FOR STRAIN IDENTIFICATION AND VACCINE PREPARATION

DAME J B; Biostematics Laboratory Animal Parasitology Institute; Beltsville Agr Res Center, Beltsville, **MARYLAND** 20705.
Proj. No.: 1265-34000-002-00D
Project Type: INHOUSE
Agency ID: ARS Period: 01 JUL 85 to 01 JUL 90

Objectives: 1) Develop a reliable system to distinguish between related strains and/or species of Trichinella, Ostertagia, Haemonchus and Eimeria based on differ-ences in the structure of their DNA; 2) clone and express in Escherichia coli, genes from Toxoplasma gondii and Eimeria spp., which encode antigens that may be useful in preparing vaccines or diagnostic tests for these parasites.

Approach: 1) Genomic DNA libraries will be prepared in plasmids of Escherichia coli and clones of repeated DNA sequences, ribosomal RNA genes and mitochondrialDNA identified. These clones will be used as DNA hybridization probes to detect strain and species differences by southern blot analysis. 2) Geno- mic DNA libraries will be prepared in the E. coli expression vector gambda gt11 by the mung bean nuclease method. Clones of genes encoding parasite antigens will be identified using antibodies. Recombinant-produced anti- gens will be compared with native antigens and their value as a vaccine component tested.

Progress: 88/01 to 88/12. Ribosomal DNA genes were cloned from Trichinella spiralis and subcloned in plasmid vectors for use as DNA probes. These subcloned fragments were useful in studying the systematics and in the characterization of T. spiralis (pig biotype) and T. pseudospiralis. A cloned DNA sequence was also identified from a T. spiralis cDNA expression library which has application in serodiagnosis. The characterization of these gene sequences provided information on the excretory-secretory antigens of this parasite. We isolated DNA from ethanol fixed cestode proglottids and determined genetic markers that support the recognition of a new species of Taenia from Taiwan. In an attempt to find an antigen for use in a vaccine against coccidiosis of chickens, DNA encoding a recombinant antigen of Eimeria acervulina merozoites was cloned after immunoscreening bacteriophage expression libraries. The purified recombinant antigen, believed to be a series of related surface proteins, stimulated T cells from Eimeria-immune chickens and when administered to susceptible chickens conferred partial protection. Sequencing of a cDNA encoding a portion of a second E. acervulina merozoite p250 surface protein revealed tandem-repeated DNA similar to structures reported for malaria. Similarities in protein structure between different protozoans of the Apicomplexa may imply similar means of evading immune responses by the host.

Publications: 88/01 to 88/12

ZARLENGA, D.S. and DAME, J.B. 1988. Molecular cloning and characterization of ribosomal RNA genes from Trichinella spiralis. The FASETS Journal 2(5):A 1028.

JENKINS, M.C. 1988. A cDNA encoding a merozoite surface protein of the protozoan Eimeria acervulina contains tandem-repeated sequences. Nucleic Acids Research. 16(20):9863.

JENKINS, M.C., DAME, J.B., LILLEHOJ, H.S., DANFORTH, H.D. and RUFF, M.D. 1988. Cloned genes coding for avian coccidiosis antigens. U.S. Patent Application, 7-155,264.

JENKINS, M.C., LILLEHOJ, H.S. and DAME, J.B. 1988. Eimeria acervulina: DNA cloning and characterization of recombinant sporozoite and merozoite antigens. Exp. Parasitol. 66:96-107.

LILLEHOJ, H.S., JENKINS, M. C., BACON, L.D., FETTERER, R.H. and BRILES, W. E. 1988. Protection against Eimeria acervulina correlates with T cell response to r ecombinant surface merozoite antigen. Exp. Parasitol. 67:148-158.

JENKINS, M.C., LILLEHOJ, H.S., DANFORTH, H.D. and FETTERER, R.H. 1988. cDNA encoding antigens of Eimeria acervulina: DNA sequence analysis; T cell and B cell epitopes. Ann. Mtg. Fed. Exp. Biol., Las Vegas, NV (ABSTRACT).

JENKINS, M.C., STROHLEIN, D.A., DANFORTH, H.D. and LILLEHOJ, H.S. 1988. Cloning of genes encoding surface antigens of Eimeria acervulina sporozoites and merozoites. Ann. Mtg. Poult. Sci. Ass., Baton Rouge, LA (ABSTRACT).

32.012 CRIS0136647
E. COLI THAT CAUSE EDEMA DISEASE: MOLECULAR ANALYSIS OF VIRULENCE MECHANISMS

O'BRIEN A D; MOON H W; Henry M. Jackson Foundation For Military Medicine; 4301 Jones Bridge Road, Bethesda, **MARYLAND** 20814.
Proj. No.: MDR-8801376 Project Type: CRGO
Agency ID: CRGO Period: 15 SEP 88 to 30 SEP 91

Objectives: PROJ. 8801376. To test two hypotheses. These hypotheses are that: a) edema disease of swine (ED) is caused by systemic distribution of an Escherichia coli cytotoxin called the Shiga-like toxin variant (SLT-IIv); and b) SLT-IIv contributes to the pathogenesis of post-weaning diarrhea of swine.

Approach: The structural genes of SLT-IIv will be cloned, sequenced, and analyzed by a computer program; the role of SLT-IIv in the pathogenesis of ED and postweaning diarrhea will be evaluated by constructing an SLT-IIv negative mutant and comparing its virulence to wild type ED strains in weaned pigs; the capacity of passively administered anti-SLT-IIv to protect pigs from ED will be assessed; the mechanisms by which ED strains colonize the bowels of pigs will be examined.

32.013 CRIS0095188
INTRODUCTION, EXPRESSION AND REGULATION OF THE PROCINE GROWTH HORMONE GENE IN SWINE

EBERT K; GOODMAN R; Anatomy & Cellular Biology; Tufts University, Boston, **MASSACHUSETTS** 02111.
Proj. No.: MASV-631113 Project Type: STATE
Agency ID: CSVM Period: 01 OCT 84 to 30 JUN 85

Objectives: To improve the rate and efficiency of meat production through methods of regulating the animal's endocrine system and metabolism. Two parallel goals will be pursued: the manipulation of genes for growth-promoting peptide hormones (porcine growth hormone) including the attachment of selective promoter sequences to amplify the expression of the gene and the introduction of these genes into porcine ova by techniques of microinjection of viral transduction.

Approach: See Objectives.

32.014 CRIS0135591
CHARACTERIZATION OF SYLVATIC AND DOMESTIC T. SPIRALIS ISOLATES BY DNA PROFILING AND ANTIGEN ANALYSIS

WORLEY D E; BURGESS D E; Veterinary Research Laboratory; Montana State University, Bozeman, **MONTANA** 59717.
Proj. No.: MONB00429Project Type: ANIMAL HEALTH
Agency ID: CSRS Period: 19 AUG 88 to 30 SEP 89

Objectives: To compare strains of Trichinella spiralis from various wild hosts and from domestic pigs using DNA sequence patterns and monoclonal antibodies.

Approach: Strains of Trichinella spiralis obtained from wild carnivores and domestic pigs will be maintained by periodic passage in Swiss Webster or deer mice. Muscle larvae reared in rodents will be collected by peptic digestion, washed in PBS, and used to prepare worm homogenates for electrophoresis and other analytical procedures, including restriction fragment length polymorphism (RFLP) analysis and polyacrylamide gel electrophoresis (SDS-PAGE). Portions of gels will be stained by Coomassie blue and silver nitrate and other portions will be used to prepare western blots. Deer mouse sera will be used to probe these blots.

Progress: 88/01 to 88/12. Six different Trichinella isolates from various sylvatic carnivores, and one isolate from a domestic pig were examined and compared by RFLP (restriction enzyme fragment length polymorphism) patterns of genomic DNA. Restriction enzyme fragments generated by the EcoRI restriction (Fig. 1) show a unique band of repetitive fragments in the Thai Pig isolate at about 1.8 kb which are not present in the sylvatic isolates. Conversely, there are unique bands in the sylvatic isolates at about 5.0 kb which are not present in the Thai Pig isolate. Patterns between 6 sylvatic isolates are nearly identical. Genomic DNA fragments generated by digestion with the Hind III restriction enzyme show unique bands in Thai Pig DNA at 1.5 kb, 1.8 kb, and 6.0 kb which are not present in the sylvatic isolates. A unique band occurs in lynx DNA at 9.8 kb which is not present in any of the other sylvatic isolates. Marked differences were also revealed in the antigens recognized between antigenic sources as well as by sera from mice infected with different strains when T. spiralis muscle larvae were separated by SDS-PAGE, electroblotted and probed with these sera.

Publications: 88/01 to 88/12
WORLEY, D.E., SEESEE, F.M. and ZARLENGA, D.S. 1988. Differential freezing resistance of T. spiralis isolates from MT & Alberta gray wolves w/observations on subspecific relationships of the MT biotype. Proc. Helm. Soc. Wash. sub.

32.015* CRIS0045857
FOOT-AND-MOUTH GENE AND ANTIGENIC STRUCTURE: EXPRESSION OF FMDV IMMUNOGENS

MOORE D M; GRUBMAN M J; KENDALL J; Agricultural Research Service; Plum Island Animal Dis Center, Orient Point, **NEW YORK** 11944.
Proj. No.: 1940-20460-045-00D
Project Type: INHOUSE
Agency ID: ARS Period: 21 NOV 79 to 30 SEP 86

Objectives: To determine the structure (sequence) of immunogens of FMDV and determine the basis for the immune response to FMDV. To

compare the variability of FMDV strains through analysis of the genome products and functions. To study and develop protein vaccines produced by chemical synthesis, through gene expression in procaryotes, eucaryotes, and in infectious virusvectors.

Approach: Identify the polypeptide sequences of virus structural and other viral encoded proteins through nucleotide sequencing of cloned viral genetic material or directly sequence selected polypeptides. Compare sequences of variants to study variation in antigenicity of FMDV. Study the basis of immunization through the preparation of experimental vaccines using poly- peptides generated by chemical synthesis, or biosynthesis in cells engineered to produce viral proteins. Immunize laboratory animals and livestock to determine immunization and protection against FMD with variouspolypeptide vaccines. Identify the location and structure of antigenic sites through competitive inhibition using selected synthetic peptides. Explore the feasibility of constructing native viral antigenic structures as vaccines through expression of viral genes in transformed cells or by viral vectored FMD genes. Study the processing of viral antigens through selective cloning techniques.--Plum Island, NY, Molec. Biol. Lab. 101-C, BL-3, 1/29/80, DM Moore/DO Morgan/MJ Grubman/JL Card/M Zellner/KH Axelson.

Progress: 86/01 to 86/12. Gene segments coding for FMDV viral protein VP-1 cloned into bacterial plasmid expression vectors synthesize high levels of the polypeptide in E. coli. Type A12 VP-1 has been extensively tested in cattle to evaluate the effectiveness of the protein vaccine. High levels of immunity were obtained in the majority of animals and reduced severity of symptoms was observed for animals which became infected on challenge of immunity. Virus type O1 VP-1 has previously generated poor immunity in livestock. Recent tests with a combined polypeptide of two areas of the VP-1 protein generated moderate protective levels in cattle vaccinated twice. In connection with antigenic analysis of VP-1 of type A and O FMDV, variants resistant to specific neutralizing monoclonal antibodies were generated. The variants are analyzed for nucleotide sequence changes in the RNA genome to pinpoint the important sites relating to immunization. The gene segment for VP-1 has been isolated and engineered into vaccinia virus as an experimental, live recombinant viral vaccine. Cell cultures infected with the recombinant virus synthesize FMDV VP-1, but vaccination of guinea pigs, cattle and mice failed to mount an antiviral response. It is likely that the cytoplasmically located VP-1 antigen was not released or that the immunogenicity of the VP-1 as presented to the host was poor. Experiments are being extended to incorporate additional structural components to facilitate morphogenesis and increase potency.

Publications: 86/01 to 86/12
NO PUBLICATIONS REPORTED THIS PERIOD.

32.016 CRIS0047777
MAPPING AFRICAN SWINE FEVER VIRUS CLONED DNA FRAGMENTS: STRUCTURAL/NONSTRUCTURAL VIRAL PROTEINS

MOORE D M; SWANEY L M; Agricultural Research Service; Plum Island Animal Dis Center, Orient Point, **NEW YORK** 11944.
Proj. No.: 1940-34000-001-00D
Project Type: INHOUSE
Agency ID: ARS Period: 20 JUL 82 to 19 JUL 87

Objectives: To locate the genes of African Swine Fever Virus (ASFV) which code for structural and non-structural immunologically reacting proteins on the genome map of the virus.

Approach: Fragments of ASFV have been cloned in expression plasmids that replicate inE. coli. Some of these fragments direct the production of ASFV proteins that react with polyclonal antiserum to ASFV. The locations of these specific fragments have been found on the genome map of ASFV. These clones will be tested for reaction with available monoclonal antibodies to identi-fy their protein products and by Western blots to determine if the productsrepresent complete or incomplete proteins. A search will be conducted amongrestriction endonuclease fragments of digests of ASFV DNA prepared from several enzymes to identify additional reacting clones, and by colony hybridizations to locate larger segments of ASFV DNA that may encode the complete protein. The larger segments will be cloned into expression vectors that permit insertion in each of the three reading frames, or into other vectors with linkers that adjust reading frames. The protein productswill be characterized as noted above.--Plum Island, NY, Molec. Biol. Lab. 101-B, BL-3, 11/23/82; DM Moore, LM Swaney, F Lyburt.

Progress: 87/01 to 87/07. African swine fever virus (ASFV) DNA Pst 1 fragments of the virulent Lisbon'60 isolate were cloned into the E. coli expression plasmid pUC9. These were tested for the expression of antigenic ASFV proteins by an immunodot blot assay with antisera from an ASF convalescent pig and subsequently with monoclonal antibodies (MCAB) to 14 ASFV proteins. The expressing ASFV inserts (19 clones) fell into 5 size categories which mapped at different conserved locations on the genetic map of the Spanish Badajoz-V isolate. One clone reacted by immunodot blot with a MCAB to viral protein 32,000; another reacting with polyclonal antiserum was identified by PAGE western blotting to express a protein of 18,000 Daltons. Several cloned Pst 1 fragments were mapped to the 4.8 Kb Eco R1 K fragment cloned from the Badajoz-V strain. Clone 40 with a 6.6 Kb insert extended into fragment K and expressed an antigenic protein of 22,000 Daltons; clone 35, 1.1 Kb, mapped within fragment K and produced an 18,000 Dalton protein; and clone 28 of 1.4 Kb mapped internally, but did not express an antigenic protein. The terminally redundant Pst 1 end fragments of the Lisbon'60 and Badajoz-V isolates have been identified by hybridization to specific cloned Eco R1 end fragments. A cloned 9.5 Kb Lisbon'60 ASFV DNA insert was

biotinylated & used successfully as probe in in situ hybridization studies. Method has been used to study location of ASFV DNA in chronically infected swine.

Publications: 87/01 to 87/07

SWANEY, L.M., LYBURT, F., MEBUS, C.A., BUONAVOGLIA, C. and ORFEI, A. 1987. Genone analysis of African Swine Fever Virus isolated in Italy in 1983. Vet. Microbiol. 14:101-104.

GALO, A.J., MEBUS, C.A. and SWANEY, L.M. 1987. In situ hybridization technique for the localization of African swine fever virus DNA. Workshop on African Swine Fever and Pig Immunology, Nov. 28-Dec. 1 (Abstract).

32.017*
CRIS0140880

PRIMARY STRUCTURE OF THE FMDV GENOME AND GENERATION OF INFECTIOUS DNA CLONES

MOORE D M; WIMMER E; VAKHARIA V; Microbiology; State University of New York, Stony Brook, **NEW YORK** 11794.
Proj. No.: 1940-34000-012-04S
Project Type: COOPERATIVE AGREE.
Agency ID: ARS Period: 01 OCT 85 to 30 SEP 87

Objectives: To develop rapid methods to sequence and study the primary structure of theFMDV genome to determine function of the genome and antigenic characteristics of capsid protein antigens. To examine the transcription and translation in vitro and the expression of cDNA segments of FMDV protein coding regions in tissue cultures. To study the processing of FMDV polyproteins and evaluate the possibility of generating native structures of the capsid proteins of the virus.

Approach: Sequence data will be obtained from the genomic RNA or cloned cDNA segmentsof the FMDV genome. Specific areas of the genome will be selected for study, with the non-coding areas of the genome, the capsid protein coding regions, and coding regions for other non-structural proteins. Specialized vectors containing protein coding cDNA segments of the FMDV genome will be transcribed in vitro and subsequently translated in vitro. Vectors will be transfected into mammalian tissue culture cells to study the expression/ assembly of the capsid region. The function of non-structural proteins and the antigenicity of capsid structural proteins will be studied in this manner. The regeneration of progeny virus from cloned cDNA will be considered by transfection of either plasmids containing full-length genomeinserts or transfection of RNA transcripts of cDNA clones.

Progress: 88/01 to 88/12. Previously, in vitro transcription/translation systems were used to determine the requirements for expression and proteolytic processing of the capsid polyprotein precursor molecule into the individual capsid proteins. To examine the processing of FMDV proteins expressed in vivo, two transient expression systems were developed. Different segments of the coding sequence of the FMDV genome were cloned into plasmids containing either the bacteriophage T7 promoter or a vaccinia late promoter. These were transfected into tissue culture cells infected with a recombinant vaccinia virus expressing the T7 RNA polymerase or with wild type vaccinia virus WR, respectively. Cells were harvested and extracts of the cells were analyzed by western blot analysis with a VP1 antiserum/125I-protein A detection system. The results showed that the P1-2A region was efficiently cleaved from precursor polyprotein and that the P1 region was further processed to capsid polypeptides if the clones contained the coding sequence for the viral protease, 3C. The results indicate that FMDV proteins expressed under the control of the T7 promoter or a vaccinia promoter can be effectively processed into capsid proteins in vivo and that stable vaccinia virus recombinants should be able to be engineered to express the same proteins. Work is underway to engineer and isolate such recombinant vaccinia viruses.

Publications: 88/01 to 88/12

VAKHARIA, V.N., DEVANEY, M.A., GRUBMAN, M.J., and MOORE, D.M. 1988. Cloning and expression of foot-and-mouth disease virus genes. XI Pan American Congress of Veterinary Sciences. Lima, Peru. (Abstract).

32.018
CRIS0097680

PIG/FETAL DEVELOPMENT: UTEROFERRIN AND MECHANISMS OF TRANSPLACENTAL IRON TRANSPORT

SIMMEN R C M; Animal Science; Ohio State University, Wooster, **OHIO** 44691.
Proj. No.: OH000816 Project Type: HATCH
Agency ID: CSRS Period: 01 JAN 86 to 31 DEC 88

Objectives: To examine the mechanisms underlying the temporal and tissue specific expression of uteroferrin (Uf), a uterine secretory glycoprotein in sow, as a first step towards the elucidation of its biological significance in fetal iron transport and fetal development.

Approach: A molecular approach using Recombinant DNA technology will be carried out to study the regulation and synthesis of Uf. Isolate and characterize the complimentary DNA (cDNA) for Uf. Isolate and characterize the chromosomal gene for Uf using the cDNA as probe. Evaluate the relative abundance of mRNA for Uf at different endocrine states of sow. Identify Uf and Uf-like genes in other species. Characterize fetal and placental receptor for Uf (f) Transfection experiments using Uf gene and BPV in mouse C127 cells or human uterine cells.

Progress: 88/01 to 88/12. The objectives of the research are to determine the mechanisms of regulation of uteroferrin, a porcine uterine iron transport protein and its role in neonatal anemia. To date we have isolated and characterized the entire chromosomal Uf gene. The gene is separated into four exons by three intervening sequences and spans approximately 6 kilobases. Exon I consists of sequenced coding for the 5' untranslated region. Exon II encodes

the initiation codon as well as the first 759 nucleotides of the Uf structural sequences. Exon III contains the sequences coding for amino acids 253 to 291 of the precursor protein while the remaining sequences of the Uf mRNA are located in Exon IV. The simplistic organization of the Uf gene contrasts with the structural pattern of the Fe-binding protein gene family characterized to date. The identification of the putative regulatory sequences within the gene will now allow for elucidating the role of the steroid hormones progesterone and estrogen in the transcriptional and post-transcriptional regulation of Uf. This in turn may provide insights into ways to manipulate Uf expression in maternal uterus which may help alleviate anemia in neonates.

Publications: 88/01 to 88/12

KETCHAM, C.M., NICK, H., SIMMEN, R.C.M. and ROBERTS, R.M. 1988. Molecular cloning of the type 5 tartrate resistant acid phosphatase from human placenta. J. Biol. Chem. (In press).

SIMMEN, R.C.M., SIMMEN, F.A., KO, Y. and BAZER, F.W. 1988. Differential growth factor content of uterine luminal fluids from Large White and prolific Meishan pigs during the estrous cycle and early pregnancy. J. Anim. Sci. (In press).

SRINIVAS, V. and SIMMEN, R.C.M. 1988. Porcine Uteroferrin Gene: Analysis of Structure and Identification of 5′ Regulatory Sequences. The American Society for Cell Biology Annual Meeting, San Francisco (Abstract).

FARMER, S.J., SIMMEN, R.C.M. and SIMMEN, F.A. 1988. Pregnancy-Associated Uterine Expression of the mRNA encoding Porcine Anti-leukoproteinase Inhibitor.

32.019 CRIS0133909

CLONING AND EXPRESSION OF THE E(2) GLYCOPROTEIN GENE OF TRANSMISSIBLE GASTROENTERITIS VIRUS

JACKWOOD D J; SAIF L J; Food Animal Health Research; Ohio State University, Wooster, **OHIO** 44691.

Proj. No.: OHO00893 Project Type: HATCH
Agency ID: CSRS Period: 01 NOV 88 to 31 OCT 91

Objectives: The objectives are: Prepare molecular clones from the genome of TGEV and identify the E2 viral protein gene; Identify open reading frames which code for the E2 envelope protein; Express the E2 viral protein using rebombinant DNA techniques and examine its antigenicity using monoclonal antibodies.

Approach: Clones of the E2 viral protein gene will be obtained using the TGEV genome and synthetic DNA primers which hybridize to the 3′ and 5′ ends of the E2 gene. The double-stranded cDNA prepared using these primers will be treated with S1 nuclease, poly-dC tailed using terminal transferase and cloned into the Pst I site of poly-dG tailed pUC9. Nucleotide sequence data will be used to identify E2 gene clones and to locate the open-reading frame of this gene. Expression of the E2 gene will be conducted in vectors which employ eukaryotic promoters.

Progress: 88/11 to 88/12. The virulent Miller strain of transmissible gastroenteritis virus (TGEV) was grown in swine testicular cell culture. The viral RNA was extracted from TGEV virions and used to prepare cDNA molecules. The cDNA was inserted into the plasmid pUC9 and then used to transform the Escherichia coli strain JM 107. The cDNA inserts observed in this library ranged in size from 500 base pairs (bp) to over 3,000 bp. Viral specific cloned gene segments were identified using colony blot hybridization and probes specific for TGEV. Clones specific for the 3′ end of the E2 glycoprotein gene were identified using a probe prepared from the clone Hpa-1600 which was provided by Dr. R. Wesley, National Animal Disease Center, USDA, Ames, IA. Clones specific for the 3′ end of the TGEV genome were identified using viral specific probes prepared in our laboratory. Nucleotide sequencing of clones representing the E2 gene is currently in progress.

Publications: 88/11 to 88/12

NO PUBLICATIONS REPORTED THIS PERIOD.

32.020 CRIS0089601

PSEUDORABIES VIRUS: MOLECULAR EPIDEMIOLOGY AND NEW APPROACHES TO ANALYSIS OF LATENT INFECTIONS.

LAWRENCE W; Pathobiology; University of Pennsylvania, Kennett Square, **PENNSYLVANIA** 19348.

Proj. No.: PENV-5-25249
Project Type: ANIMAL HEALTH
Agency ID: CSRS Period: 09 FEB 83 to 30 SEP 85

Objectives: Evaluate the usefulness of restriction enzyme analysis of pseudorabies virus (PrV) DNA (fingerprinting) for identification of vaccine and field strain of PrV. Determine whether correlations can be made between different viral strains and biilogical characteristics such as latency, virulence, and clinical forms of disease. Through collaborative efforts with other investigators, utilize DNA fingerprinting for epidemiologic studies which seek to determine the number of strains which may exist in individual herds, within defined geographical regions and when appropriate isolations are made, to trace sources of infection.

Approach: Employ Southern blot DNA hybridization techniques to detect latent PrV genomes in tissues of seropositive animals. Use these methods to assess the occurrence of latent infections and to determine tissue sites which harbor latent virus.

Progress: 85/10 to 86/09. Restriction enzyme Hind III DNA fragments representing the entire BHV1 genome were cloned in the plasmid pBR322. A BHV1 monoclonal antibody resistant mutant (mar-6) was isolated by selecting for resistance to a neutralizing monoclonal antibody directed against a 130,000 Dalton viral glycoprotein (g 130). The mutation in mar-6 was mapped by a marker rescue technique, utilizing cloned fragments of the BHV1 genome, to a 3.8 kilobase DNA fragments at map units

0.405 - 0.432. Thus, the site of the mutation in mar 6, and perhaps the entire gene coding for g130, lies within this 3.8 kb DNA fragment. Utilizing cloned fragments of the BHV1 genome, the gene for the BHV1 specified thymidine kinase has been mapped to a position within the viral Hind III A DNA fragment at approximate map position 0.46.

Publications: 85/10 to 86/09

LAWRENCE, W.C., D'URSO, R.C., KUNDEL, C.A., WHITBECK, J.D. and BELLO, L.J. Map loction of the gene for 130,000 Dalton glycoprotein of bovine herpesvirus 1. Eleventh International Herpesvirus Workshop, Leeds, England, 1986. p. 302.

LAWRENCE, W.C., D'URSO, R.C., KUNDEL, C.A., WHITBECK, J.C. and BELLO, L.J. 1986. Map location of the gene for 130,000 Dalton glycoprotein of bovine herpesvirus 1. J. Virol. 60: 405-414.

32.021* CRIS0097149

GENETIC ORGANIZATION AND FUNCTION IN THE PRODUCTION OF THE F41 BACTERIAL ADHESIN

MOSELEY S L; Microbiology; University of Washington, Seattle, **WASHINGTON** 98195.
Proj. No.: WNR-8502362 Project Type: CRGO
Agency ID: CRGO Period: 01 SEP 85 to 31 AUG 87

Objectives: Proj 8502362. The proposed research seeks to characterize the genetic basis for the production of E. coli adhesins F41 by enterotoxigenic E. coli. The plasmid or chromosomal location of the gene will be determined, and the number and function of gene products will be analyzed. The genetic relatedness of F41 with other E. coli adhesins will be determined. The nucleotide sequence of the DNA encoding the F41 structural subunit will be determined. A specific hybridization probe for the detection of strains of E. coli which product F41 by colony DNA hybridization will be developed.

Approach: The cloned F41 gene will be physically mapped by restriction analysis. The gene will be localized by analysis of transposon Tn5 insertion mutants. The Tn5 insertion mutants, as well as the wild type cloned gene, will be analyzed for expression of products in an E. coli minicell system. Function of the various products will be determined by phenotypic expression by the insertion mutants of antigen and hemagglutination properties. Genetic relatedness will be studied by Southern blot hybridization of F41-encoding DNA with DNA encoding other adhesins of E. coli. An appropriate restriction fragment hybridizing only to the F41 gene will be developed as an F41 specific hybridization probe.

Progress: 87/01 to 87/08. Genes encoding the production of the F41 bacterial adhesin have been isolated from an enterotoxigenic Escherichia coli strain pathogenic for pigs, and characterized. The determminant was found by Southern blot hybridization to be chromosomal in all F41-producing strains exmined. Four gene products were identified by E. coli maxicell analysis to be associated with F41 production, ad two additional genes were identified by nucleotide sequence analysis. The genes were physically mapped. The entire DNA region encoding F41 was found to share extensive homology with the K88 determinant of E. coli, with the exception of the regions encoding the fimbrial subunits which were non-homologous. The genetic organization of F41 was very similar to that of K88. Nucleotide sequence analysis of the structural subunit gene of F41 predicted a product with structural similarities to K88 and other E. coli fimbriae. K88 and F41 specific hybridization probes were derived from the structural subunit genes of each determinant. Hybridization probes were used to examine a number of E. coli isolates from animals and humans. Some animal isolates reacting with probes derived from sequences shared by K88 and F41 determinants produced neither K88 nor F41. Several of these strains produced fatal septicemia in newborn colostrum-deprived pigs. The probes also detected human enteroinvasive E. coli isolates, and the homology was localized to the large virulence-associated plasmid of these strains.

Publications: 87/01 to 87/08

MOSELEY, S.L., DOUGAN, G., SCHNEIDER, R.A. and MOON, H.W. 1986. Cloning of chromosomal DNA encoding the F41 adhesin of enterotoxigenic Escherichia coli and genetic homology between adhesins F41 and K88. J. Bacteriol. 167:799-804.

RUNNELS, P.L., MOSELEY, S.L., MOON, H.W. 1987. F41 pili as protective antigens of enterotoxigenic Escherichia coli that produce F41, K99, or both pilus antigens. Infect. Immun. 55:555-558.

32.022 CRIS0138491

MOLECULAR AND SOMATIC CELL GENETIC ANALYSIS OF THE SWINE GENOME

GILES R E; College of Vet Medicine; Washington State University, Pullman, **WASHINGTON** 99164.
Proj. No.: WNP00847 Project Type: ANIMAL HEALTH
Agency ID: CSRS Period: 01 JUL 89 to 30 JUN 92

Objectives: Assign genetic markers to positions on swine chromosomes by in situ nucleic acid hybridization using cloned probes for specific genes or anonymous DNA sequences. Produce a clone panel of interspecific somatic cell hybrids between swine cells and rodent cells for gene mapping via somatic cell and molecular genetics. Establish stocks of hybrid cells for swine genetics. Characterize the clone panel for swine chromosomes, the presence of specific swine nucleic acid sequences, and swine isozymes. Screen swine breeds for RFLPs of use in genetic analysis of quantitative trait loci (QTLs; Lander & Botstein, Genet. 121:185-199, 1989).

Approach: Cytogenetic studies and in situ nucleic acid hybridization will be performed using Giemsa banding, Hoechst 33258 staining, Quinicrine staining, autoradiography with H-thymidine, and avidin-biotin labelling. Interspecific somatic cell hybrids will be produced by polyethylene glycol fusion of

rodent cells to diploid swine fibroblasts or peripheral blood lymphocytes and isolated by biochemical selection. Isozyme analysis will be performed by electrophoretic methods. RFLPs will be detected by the Southern technique.

33.001 CRIS0130003
BLUETONGUE VIRUS MORPHOGENSIS USING RECOMBINANT DNA TECHNOLOGIES

POLLY R; Environ Health Sciences; University of Alabama, Birmingham, **ALABAMA** 35294.
Proj. No.: ALAR-8600991 Project Type: CRGO
Agency ID: CRGO Period: 01 SEP 86 to 31 DEC 87

Objectives: PROJECT 8600991. The purpose of this project as to elucidate the molecular biology of bluetongue virus (BTV) and thereby determine the basis for its disease and transmission potential. Bluetongue is an important sheep and cattle disease in the United States (U.S.), Australia, India, Africa and South America.

Approach: Both molecular and genetic approaches will be used to develop the proposed study which will focus on understanding the viral specific processes induced in infected cells, in particular the mechanism of self-assembly and RNA replication. Expected benefits to result from the program include the development of bluetongue subunit vaccines for animal protection.

Progress: 87/01 to 87/12. Full length DNA clones representing the 10 double-stranded RNA segments of US bluetongue virus serotype 10 (BTV-10) have been used in a Northern blot hybridization study to determine the genetic relationships among 20 different BTV serotypes. The results obtained indicate that all the genes representing the nonstructural proteins (NS1, NS2 and NS3) and most of the inner capsid proteins are highly conserved (e.g., VP1, VP3, VP4) while VP6 and VP7, the remaining two are less conserved. The genes representing the two outer capsid proteins, VP2 and VP5, vary significantly. Hybridization studies with complete clones of type specific gene, segment 2, of 5 US serotypes (2, 10, 11, 13, 17) and one Australian serotype (BTV-1) have indicated that despite geographical distances, a certain BTV serotype exhibit similarities. To determine the extent and nature of genetic variation between five US serotypes of BTV, the complete nucleotide sequences were determined from cDNA clones representing the L2 dsRNA of each serotype (BTV-2, -10, -11, -13 and -17), the gene that codes for the outer capsid neutralization antigen (VP2). The predicted amino acids of five proteins were then compared with each other. The results indicated that VP2 proteins of BTV-10, -11 and -12 are 70% homologous to each other, while VP2 of BTV-13 and BTV-2 are only 40% homologous with these three serotypes, although they exhibit 47% homology with each other.

Publications: 87/01 to 87/12

GHIASI, H. FUKUSHO, A., ESHITA, Y. and ROY, P. Identification and characterization of conserved and variable regions in the neutralization gene of bluetongue virus. Virology, 160: 100-109, 1987.

INUMARU, S., GHIASI, H. and ROY, P. Expression of bluetongue virus group specific antigen VP3 in insect cells by a baculovirus expression vector: its use for detection of bluetongue virus antibodies. J. Gen Virol., 68:1627-1637.

FUKUSHO, A., RITTER, G.D. and ROY, P. Variation in the bluetongue virus neutralization protein VP2. J. Gen. Virol., 68:2967-2973, 1987.

YU, Y., FUKUSHO, A. and ROY, P. Nucleotide sequence of the VP4 core protein gene (M4 RNA) of U.S. bluetongue virus serotype 10. Nucleic Acids Research, 15:7206, 1987.

LEE, J. and ROY, P. Complete sequence of the NS1 gene (M6 RNA) of U.S. bluetongue virus serotype 10. Nucleic Acids Research, 15:7207, 1987.

YAMAGUCHI, S., FUKUSHO, A. And ROY, P. Complete sequence of neutralization protein VP2 of the recent US isolate Bluetongue virus serotype 2: its relationship with VP2 species of other US serotypes. (Submitted).

YAMAGUCHI, S., FUKUSHO, A. and ROY, P. Complete sequence of VP2 gene of the bluetongue virus serotype 1 (BTV-1). Nucleic Acid Research (submitted).

33.002 CRIS0137347
MOLECULAR GENETICS OF SCRAPIE IN SHEEP AND COWS

WESTAWAY D A; Neurology; University of California, San Francisco, **CALIFORNIA** 94143.
Proj. No.: CALR-8801464 Project Type: CRGO
Agency ID: CRGO Period: 01 AUG 88 to 31 JUL 90

Objectives: PROJ. 8801464. Our objectives are to isolate and characterize the prion protein (PrP) genes in livestock animals and to relate these to loci determining susceptibility to scrapie disease. These objectives, as well as our intended experimental APPROACH are discussed in detail in our full grant application.

Approach: In brief, we will obtain molecular of PrP genes from a Suffolk sheep. The nucleotide sequences of these clones will define antigenic peptides against which antisera may be raised. These clones, as well as ovine histocompatibility antigen clones, will be used for genetic linkage analysis of a predisposition locus in familial scrapie. If linkage to the PrP gene is established, we will sequence the PrP genes from multiple affected sheep (recovered via the polymerase chain reaction) in an attempt to define diagnostic oligonucleotide probes to detect base substitutions.

33.003* CRIS0138576
IMMUNOLOGY AND MOLECULAR BIOLOGY OF ECTOPARASITIC MITES

BOYCE W M; Veterinary Microbiology Immuno; University of California, Davis, **CALIFORNIA** 95616.
Proj. No.: CA-V*-VMB-5093-H Project Type: HATCH - PENDING
Agency ID: CSRS Period: 11 JUL 89 to 30 SEP 93

Objectives: To develop an immunoassay for diagnosis of mite infestations, determine the genetic relatedness of mites using molecular

analysis of parasite DNA; and conduct epidemiologic studies of host specificity and treatment strategies.

Approach: Immunoassays will be developed using antigens extracted from mites and sera from naturally and experimentally infected animals. Cross transmission studies will be performed by transferring mites onto new hosts under experimental conditions. Genetic relatedness will be determined through a variety of molecular biology techniques including RFLP and DNA hybridization.

33.004* CRIS0131805
FACTORS INFLUENCING EMBRYO SURVIVAL IN DOMESTIC FARM ANIMAL SPECIES

FORD S P; Animal Science; Iowa State University, Ames, **IOWA** 50011.
Proj. No.: IOW02825 Project Type: HATCH
Agency ID: CSRS Period: 01 JUL 87 to 30 SEP 91

Objectives: Investigate the effect of SLA haplotype on the rate of development and survival of pig embryos, and develop plans to improve litter size by identifying SLA haplotypes of breeding pairs. Continue investigations on hormonal control of uterine blood flow throughout pregnancy in the ewe, cow and sow, with emphasis on the role of the uterine vasculature in conceptus growth and survival. In vitro perfusion of the bovine placentome as a "mini placenta" to study the factors which control the flow of blood to the fetal-maternal interface in the cow as well as factors which control bovine placental steroid production.

Approach: Identify SLA haplotypes in pigs by restriction fragment length polymorphism analysis. Relate these SLA haplotypes to developmental rate of embryos by: identification of SLA antigens on very early pig embryos utilizing an enzyme-linked immunosorbent assay (ELISA), counting the number of blastomeres of preimplantation pig embryos of different SLA haplotypes during defined periods during early pregnancy, and evaluation of the relative survival of embryos of different SLA haplotypes. Use chronic in vivo measurement of uterine blood flow (electromagnetic flow probes, microspheres, etc.), and in vitro perfusion of the bovine placentome, to investigate factors which control flow through the uterine and placental vasculatures throughout the periods of embryo and fetal development.

Progress: 88/01 to 88/12. Effect of PGF(subscript 2)(Alpha) on porcine corpora lutea (CL) following administration on day 9 of the estrous cycle. During the period prior to day 12 of the estrous cycle, porcine CL refractory to the luteolytic effects of PGF(subscript 2)(Alpha). We investigated functional and structural aspects of the effects of PGF(subscript 2)(Alpha) on porcine CL during the refractory period. Gilts were unilaterally ovariectomized on day 8 and utero-ovarian venous (UOV) and femoral arterial (FA) catheters were inserted. Gilts received 20 mg PGF(subscript 2)(Alpha) or vehicle on day 9 and the remaining ovary was removed on day 12. Progesterone declined markedly in the FA (3 hrs) and UOV (2 hrs) following PGF(subscript 2)(Alpha), but not vehicle, and had returned to pretreatment levels by day 11. Luteal growth (weight, protein and DNA content) continued in pigs from day 8 to day 12 and was not affected by a luteolytic dose of PGF(subscript 2)(Alpha) on day 9. These data suggest that PGF(subscript 2)(Alpha) administration on day 9 of the estrous cycle in pigs has transient inhibitory effects on luteal function without effects on luteal composition. Effect of intraluteal estradiol-17(Beta) implants on weight and progesterone secretion of porcine corpora lutea (CL). Estradiol-17(Beta) (E(subscript 2)) decreases the effectiveness of prostaglandin F(subscript 2)(Alpha) (PGF(subscript 2)(Alpha)) to induce luteolysis, and E(subscript 2) locally increases CL wt in pigs suggesting a direct luteotropic effect.

Publications: 88/01 to 88/12

GUENTHER, A.E., CONLEY, A.J., VAN ORDEN, D.E., FARLEY, D.B. and FORD, S.P. (1988). Changing structural and mechanical properties of uterine arteries during porcine gestation. J. Anim. Sci. 66:3144-3152.

FORD, S.P., SCHWARTZ, N.K., ROTHSCHILD, M.F., CONLEY, A.J. and WARNER, C.M. (1988). Influence of SLA haplotype on preimplantation embryonic cell number in miniature pigs. J. Reprod. Fert. 84:99-104.

LUND, J., FAUCHER, D.J., FORD, S.P., PORTER, J.C., WATERMAN, M.R. and MASON, J.I. (1988). Developmental expression of bovine adrenocortical steroid hydrolases: regulation of P-450(subscript 17)(Alpha) expression leads to episodic fetal cor.

CONLEY, A.J. and FORD, S.P. (1989). Effects of a phorbol ester (TPA), calcium ionophore (A23187) and prostaglandin F(subscript 2)(Alpha) (PGF(subscript 2)(Alpha)) on progesterone secretion by dispersed ovine luteal cells. Biol. Reprod.

CONLEY, A.J. and FORD, S.P. (1989). Direct luteotropic effect of oestradiol-17(Beta) on porcine corpora lutea. J. Reprod. Fert. (In press.).

CONLEY, A.J., PUSATERI, A.E. and FORD, S.P. (1989). Effects of prostaglandin F(subscript 2)(Alpha), (PGF(subscript 2)(Alpha)) on porcine corpora lutea (CL) following administration on day 9 or the estrous cycle. Proceedings Midwestern Sect.

33.005 CRIS0138095
SHEEP MAJOR HISTOCOMPATIBILITY SYSTEM RELATIONSHIP TO SUSCEPTIBILITY TO NEMATODES

MILLER J E; STEAR M J; BABATUNDE K O; Animal Science; Louisiana State University, Baton Rouge, **LOUISIANA** 70803.
Proj. No.: LAB02756 Project Type: SPECIAL GRANT
Agency ID: CSRS Period: 01 JUL 89 to 30 JUN 91

Objectives: To investigate the role of the major histocompatibility system in identifying a genetic basis for host resistance to Haemonchus contortus within and between different breeds (Red Masai, Louisiana Native, and Suffolk) of sheep.

Approach: Restriction fragment length polymorphism (RFLP) and microlymphocytotoxicity testing (MLCT) techniques will be used to define the major histocompatibility system in sheep. Appropriate samples (sera, DNA, parasites, etc.) will be collected from the study populations and processed accordingly to help identify a genetic basis for differences in breeds of sheep resistant and susceptible to nematode parasitism. Half-sibling families will be produced by mating selected rams to a number of selected ewes. Data (RFLP, MLCT, parasitism, etc.) from parents and offspring will be analyzed to: determine if major genes are present; estimate the heritability; examine the effect of particular genes on parasitological parameters; and assess the interactions between particular genes, parasitism, and weight gain.

33.006* CRIS0049916

CLONING DNA OF COCCIDIAN AND HELMINTH PARASITES FOR STRAIN IDENTIFICATION AND VACCINE PREPARATION

DAME J B; Biostematics Laboratory Animal Parasitology Institute; Beltsville Agr Res Center, Beltsville, **MARYLAND** 20705.
Proj. No.: 1265-34000-002-00D
Project Type: INHOUSE
Agency ID: ARS Period: 01 JUL 85 to 01 JUL 90

Objectives: 1) Develop a reliable system to distinguish between related strains and/or species of Trichinella, Ostertagia, Haemonchus and Eimeria based on differ-ences in the structure of their DNA; 2) clone and express in Escherichia coli, genes from Toxoplasma gondii and Eimeria spp., which encode antigens that may be useful in preparing vaccines or diagnostic tests for these parasites.

Approach: 1) Genomic DNA libraries will be prepared in plasmids of Escherichia coli and clones of repeated DNA sequences, ribosomal RNA genes and mitochondrialDNA identified. These clones will be used as DNA hybridization probes to detect strain and species differences by southern blot analysis. 2) Geno- mic DNA libraries will be prepared in the E. coli expression vector gambda gt11 by the mung bean nuclease method. Clones of genes encoding parasite antigens will be identified using antibodies. Recombinant-produced anti- gens will be compared with native antigens and their value as a vaccine component tested.

Progress: 88/01 to 88/12. Ribosomal DNA genes were cloned from Trichinella spiralis and subcloned in plasmid vectors for use as DNA probes. These subcloned fragments were useful in studying the systematics and in the characterization of T. spiralis (pig biotype) and T. pseudospiralis. A cloned DNA sequence was also identified from a T. spiralis cDNA expression library which has application in serodiagnosis. The characterization of these gene sequences provided information on the excretory-secretory antigens of this parasite. We isolated DNA from ethanol fixed cestode proglottids and determined genetic markers that support the recognition of a new species of Taenia from Taiwan. In an attempt to find an antigen for use in a vaccine against coccidiosis of chickens, DNA encoding a recombinant antigen of Eimeria acervulina merozoites was cloned after immunoscreening bacteriophage expression libraries. The purified recombinant antigen, believed to be a series of related surface proteins, stimulated T cells from Eimeria-immune chickens and when administered to susceptible chickens conferred partial protection. Sequencing of a cDNA encoding a portion of a second E. acervulina merozoite p250 surface protein revealed tandem-repeated DNA similar to structures reported for malaria. Similarities in protein structure between different protozoans of the Apicomplexa may imply similar means of evading immune responses by the host.

Publications: 88/01 to 88/12

ZARLENGA, D.S. and DAME, J.B. 1988. Molecular cloning and characterization of ribosomal RNA genes from Trichinella spiralis. The FASETS Journal 2(5):A 1028.

JENKINS, M.C. 1988. A cDNA encoding a merozoite surface protein of the protozoan Eimeria acervulina contains tandem-repeated sequences. Nucleic Acids Research. 16(20):9863.

JENKINS, M.C., DAME, J.B., LILLEHOJ, H.S., DANFORTH, H.D. and RUFF, M.D. 1988. Cloned genes coding for avian coccidiosis antigens. U.S. Patent Application, 7-155,264.

JENKINS, M.C., LILLEHOJ, H.S. and DAME, J.B. 1988. Eimeria acervulina: DNA cloning and characterization of recombinant sporozoite and merozoite antigens. Exp. Parasitol. 66:96-107.

LILLEHOJ, H.S., JENKINS, M. C., BACON, L.D., FETTERER, R.H. and BRILES, W. E. 1988. Protection against Eimeria acervulina correlates with T cell response to r ecombinant surface merozoite antigen. Exp. Parasitol. 67:148-158.

JENKINS, M.C., LILLEHOJ, H.S., DANFORTH, H.D. and FETTERER, R.H. 1988. cDNA encoding antigens of Eimeria acervulina: DNA sequence analysis; T cell and B cell epitopes. Ann. Mtg. Fed. Exp. Biol., Las Vegas, NV (ABSTRACT).

JENKINS, M.C., STROHLEIN, D.A., DANFORTH, H.D. and LILLEHOJ, H.S. 1988. Cloning of genes encoding surface antigens of Eimeria acervulina sporozoites and merozoites. Ann. Mtg. Poult. Sci. Ass., Baton Rouge, LA (ABSTRACT).

33.007 CRIS0136174
CHARACTERIZATION OF THE SYNTHESIS AND STRUCTURE OF OVINE PLACENTAL LACTOGEN

ANTHONY R V; MOFFATT R J; Animal Science; University of Minnesota, St Paul, **MINNESOTA** 55108.
Proj. No.: MO-00431-1 Project Type: CRGO
Agency ID: CRGO Period: 01 SEP 88 to 31 AUG 91

Objectives: PROJ. 8801311. The objective of this research is to investigate the structure and biosynthesis of ovine placental lactogen.

Approach: Ovine placental lactogen will be purified by conventional biochemical techniques then subjected to amino acid sequencing. Placental tissue obtained at 100 days of gestation will be used as the source of mRNA to generate a cDNA library. Ovine PL cDNA's will be subjected to nucleotide sequencing in order to infer the primary amino acid sequence of oPL. Ovine PL cDNA's will also be used to analyze oPL mRNA levels and transcription rates during pregnancy.

Progress: 88/09 to 88/12. OBJECTIVE: The objective of this project is to characterize the biosynthesis, structure and regulation of ovine placental lactogen (oPL). APPROACH: The placenta not only functions as an organ for nutrient transfer from the maternal to fetal system but also functions as a site of secretory protein production. Ovine placental lactogen (oPL) is a placental secretory protein that has been purified previously and implicated as playing a regulatory role in fetal metabolism. However, little is known about its structural properties or how it is regulated. Our approach is to use fetal cotyledons from day 100 of pregnancy to purify oPL to homogeneity and also for oPL mRNA isolation. Purified oPL will be subjected to gas-phase amino acid sequencing to obtain at least a partial amino acid sequence for oPL. Fetal cotyledonary mRNA will be used to generate and isolate "full-length" cDNA's for oPL which can be nucleotide sequenced. Once a complete nucleotide sequence is obtained the complete amino acid sequence of oPL will be inferred and compared to other placental lactogens and protein hormones for similarity. With the availability of a DNA probe for oPL mRNA, the quantity of cellular mRNA and the rate of mRNA transcription for oPL will be determined throughout pregnancy and related to maternal and fetal serum concentrations of oPL. The long term goal of this research is to study the regulatory control of oPL. PROGRESS: Ovine PL has been purified to homogeneity using a variety of chromatographic procedures.

Publications: 88/09 to 88/12
No publications reported this period.

33.008 CRIS0132324
CLONING AND CHARACTERIZATION OF OVINE IGF-1 FOR TRANSFER INTO EMBRYOS

WONG E A; HUNTER A G; Animal Science; University of Minnesota, St Paul, **MINNESOTA** 55108.
Proj. No.: MIN-16-070 Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 87 to 30 SEP 90

Objectives: The objectives of this proposal are to: isolate and characterize the ovine gene for insulin-like growth factor-1 (IGF-1); construct plasmid vectors containing the ovine IGF-1 gene fused to heterologous promoters for transfer into sheep embryos; & develop a mammalian cell culture system for site directed integration.

Approach: The ovine gene for IGF-1 will be isolated from sheep liver genomic and cDNA libraries. Plasmids containing the ovine IGF-1 gene linked to heterologous promoters will be microinjected into fertilized sheep ova to generate transgenic sheep. Microinjection of DNA into cultured mammalian cells will also be performed to try to develop a system for directing a DNA sequence to a precise chromosomal site.

Progress: 88/01 to 88/12. The somatomedins or insulin-like growth factors (IGF-I and IGF-II) are small peptide hormones which are structurally related to insulin. IGF-I is thought to act as the major mediator through which growth hormone exerts its biological effects and therefore plays a key role in regulating postnatal mammalian growth. IGF-II may play a comparable role during fetal development. We have cloned and partially sequenced ovine cDNAs encoding IGF-I after screening a lamb liver cDNA library with a rat IGF-I cDNA probe. The deduced amino acid sequence for the mature, 70 amino acid ovine peptide is highly homologous to the human peptide. In addition, as has been demonstrated in other mammalian species, a variety of messenger RNAs encoding ovine IGF-I are found which differ in their 5' exons and 3' extensions. The role for these multiple transcripts has yet to be determined. We have also isolated from a lamb liver genomic library IGF-I clones containing both intron and exon sequences. We are interested in determining the genomic organization and identifying the sequences which regulate expression of the IGF genes.

Publications: 88/01 to 88/12
WHEATON, J.E., AL-RAHEEM, S.N., GODFREDSON, J.A., DORN, J.M., WONG, E.A., VALE, W., RIVIER, J., MOWLES, T.F., HEIMER, E.P. and FELIX, A.M. 1988. Subcutaneous infusion of growth hormone-releasing factors in steers and wethers.

33.009 CRIS0136665
CLONING AND CHARACTERIZATION OF OVINE INSULIN-LIKE GROWTH FACTORS

WONG E A; Animal Science; 1919 University Avenue, St Paul, **MINNESOTA** 55104.
Proj. No.: MIN-16-069 Project Type: CRGO
Agency ID: CRGO Period: 01 SEP 88 to 31 AUG 90

Objectives: PROJ. 8801487. This proposal aims to clone and characterize the genomic organization and mRNAs for ovine insulin-like growth factors I and II.

Approach: Lamb liver cDNA and genomic libraries will be constructed and screened for recombinant clones encoding the genes for ovine insulin-like growth factors I and II (IGF-I and II). Isolated clones will be identified as IGFs by DNA sequence analysis. Once cloned, the IGF cDNAs will be used as hybridization probes to investigate expression of the IGF genes at the mRNA level. Potential differential expression of IGF mRNAs will be examined in liver tissues obtained from control and growth hormone releasing factor treated lambs.

Progress: 88/09 to 88/12. The somatomedins or insulin-like growth factors (IGF-I and IGF-II) are small peptide hormones which are structurally related to insulin. IGF-I is thought to act as the major mediator through which growth hormone exerts its biological effects and therefore plays a key role in regulating postnatal mammalian growth. IGF-II may play a comparable role during fetal development. We have cloned and partially sequenced ovine cDNAs encoding IGF-I after screening a lamb liver cDNA library with a rat IGF-I cDNA probe. The deduced amino acid sequence for the mature, 70 amino acid ovine peptide is highly homologous to the human peptide. In addition, as has been demonstrated in other mammalian species, a variety of messenger RNAs encoding ovine IGF-I are found which differ in their 5' exons and 3' extensions. The role for these multiple transcripts has yet to be determined. We have also isolated from a lamb liver genomic library IGF-I clones containing both intron and exon sequences. We are interested in determining the genomic organization and identifying the sequences which regulate expression of the IGF genes.

Publications: 88/09 to 88/12
WHEATON, J.E., AL-RAHEEM, S.N., GODFREDSON, J.A., DORN, J.M., WONG, E.A., VALE, W., RIVIER, J., MOWLES, T.F., HEIMER, E.P. and FELIX, A.M. 1988. Subcutaneous infusion of growth hormone-releasing factors in steers and wethers.

33.010 CRIS0097239
GENETIC AND MOLECULAR ANALYSIS OF DRUG RESISTANCE IN CAENORHADITIS ELEGANS

CHALFIE M; Biological Sciences; Columbia University, New York, **NEW YORK** 10027.
Proj. No.: NYR-8502283 Project Type: CRGO
Agency ID: CRGO Period: 01 SEP 85 to 31 AUG 87

Objectives: Proj 8502283. We wish to use the nematode Caenorhabditis elegans to study the genetics and molecular biology of drug resistance in nematodes. Since many agents used in agriculture are broad-spectrum anthelmintics, it is likely that research on C. elegans, whose genetics and development are well understood, will be applicable to parasitic nematodes that cause considerable agricultural devastation.

Approach: The major thrust of our experiments will be on resistance to benomyl, an antimicrotubule drug. We have isolated a number of benomyl-resistant strains. We will use these to study the types of genetic abnormalities resulting in drug-resistance. Some of the mutations have resulted from transposition events and, thus should allow us to clone the gene conveying resistance. We will use such clones to examine DNA from resistant, parasitic strains to ascertain the mechanism of drug-resistance in the wild.

Progress: 87/01 to 87/12. We have studied the genetic basis of resistance to the antimicrotubule drug benomyl in the free-living nematode Caenorhabditis elegans. Twenty chemically induced mutations and three spontaneous mutations arising from a mutator strain all map to the same genetic locus, the ben-1 gene on chromosome III. The mutations are all unusual in that they result in dominant resistance. The spontaneous mutants have been used to clone the gene. Two of the spontaneous strains show altered restriction fragments when probed at low stringency with a probe for nematode beta-tubulin suggesting that the ben-1 gene encodes a beta-tubulin. We have cloned this gene and have used our probed sequences to characterize the mutants. One of the spontaneous alleles appears to be deleted for this gene. The high rate of dominant mutations as well as this finding of this deletion mutant makes it likely that the ben-1 gene encodes a dosage-dependent beta-tubulin that normally conveys sensitivity to benomyl and other benzimidazole carbamates.

Publications: 87/01 to 87/12
CHALFIE, M., DEAN, E., REILLY, E., BUCK, K. and THOMSON, J.N. 1986. Mutations affecting mictotubule structure in Caenorhabditis elegans. J. Cell Suppl. 5: 257-271.

33.011 0097881
BLUETONGUE AND EPIZOOTIC HEMORRHAGIC DISEASES IN RUMINANTS

COLLISSON E W; LIVINGSTON C W; Veterinary Microbiology & Parasitology; Texas A&M University, College Station, **TEXAS** 77843.
Proj. No.: TEX06808 Project Type: HATCH
Agency ID: CSRS Period: 01 FEB 86 to 31 JAN 91

Objectives: To develop recombinant DNA probes for bluetongue (BT) and epizootic hemorrhagic disease (EHD) viruses. To develop techniques for using these probes as a practical diagnostic tool for the clinical laboratory. To use these probes to study the pathogenesis of these viruses in the acutely infected chick

embryo, sheep, and acutely and persistently infected cattle. To study the molecular epizoology of BTV and EHDV. To determine the indigenous serotypes of BTV and EHDV in Texas for future control programs. To determine gene assignments of EHDV.

Approach: Recombinant DNA techniques will be used to develop probes for detection of BTV and EHDV genomes in infected tissues. Characterization of viral genomes will be done by such techniques as polyacrylamide gel electrophoresis (PAGE) and oligonucleotide fingerprinting, whereas proteins will be analyzed by HPLC in addition to PAGE. The extent of the diversity of BTV in Texas will be determined by monitoring seroconversion of sentinel cattle and doing retrospective isolation of the viral strains. Genome reassortment procedures, as well as in vitro translation techniques will be used to map EHDV genes.

Progress: 88/01 to 88/12. In situ cytohybridization was used to determine the tissue tropism and target cells for replication of bluetongue virus (BTV) in the developing chicken embryo. Hybridization with a biotinylated probe detected viral replication in embryos inoculated with the four U.S. serotypes and a BTV field strain. At the final stages of infection, when the embryos were hemorrhagic, viral infection could consistently be detected in the brain, kidney, spinal cord, heart, lung and liver with the brain and kidney most severly affected. Other tissues, such as the retina, skin, tongue and intestinal villi also supported viral replication with greater concentration of virus localized with in epithelial cells, such as those lining the kidney tubules and tertiary bronchi of the lungs. Within 24 hours after inoculation, viral replication occurs initially in the brain and kidney. By 48 hours, viral replication can also be detected in the lungs, heart and spinal cord with the liver being severely infected by 72 hours. Low levels of hybridization could be detected in embryos infected with EHDV.

Publications: 88/01 to 88/12

WANG, L., KEMP, M., ROY, P. and COLLISSON, E. 1988. Tissue tropsim and target cells of bluetongue virus in the chicken embryo. J. Virol. 62: 887-893.

COLLISSON, E., WANG, L., ROY, P. and KEMP, M. 1988. Detection of BTV in infected chicken embryos by in situ hybridization in orbiviruses and birnaviruses in the Proc. Double Stranded RNA Virus Symposium.

33.012 CRIS0135990

DIAGNOSTIC PROBE FOR THE SPIDER LAMB SYNDROME GENE IN SUFFOLK SHEEP

BUNCH T D; MACIULIS A; WOODWARD S; Animal Dairy & Vet Science; Utah State University, Logan, **UTAH** 84322.

Proj. No.: UTA00153 Project Type: ANIMAL HEALTH Agency ID: CSRS Period: 01 OCT 88 to 30 JUN 93

Objectives: To develop and to make available a gene probe that can be used to reduce the frequency and/or eliminate the Spider Lamb syndrome (SLS) gene from the Suffolk genome.

Approach: Identify restriction fragment-length polymorphisms RFLPs that are unique to the Suffolk breed. RFLPs from the Suffolk will be compared to four other breeds (Columbia, Hampshire, Rambouilett, and St. Croix). Six animals will be examined from each breed. Nine different restriction enzymes will be used in identifying RFLP similarities and differences within and between breeds. DNA will be isolated from the nuclei of white blood cells. A Suffolk DNA genomic library will be established using the Cosmid vector. The library will be surveyed using human tandem repeat markers (VNTR) to identify highly polymorphic RFLPs that are unique to the Suffolk genome. Once the highly polymorphic RFLPs have been identified they will be scrutinized by comparing them to the unique Suffolk RFLPs as determined in step 1. RFLP markers from the results of steps 1 and 2 will be used to screen Spider lambs and their sires and dams to determine the mode of inheritance of the RFLP markers.

Progress: 88/01 to 88/12. Techniques were learned that allow the application of human molecular genetic techniques to sheep. Eleven spider lambs were obtained, necropsied and positively identified as true spider lambs. Liver samples were collected from the lambs and stored in liquid nitrogen (-191 degrees C). Procedures are being developed to extract the DNA from the samples. Blood samples (50 cc) were collected from 30 sheep representing 5 breeds (6 each, Suffolk, Rambouilett, St. Croix, Hampshire and Targhee). DNA was extracted and purified from leucocytes. Blood samples were also collected from a few known spider lamb syndrome families. Alliquots of DNA from the 30 sheep were digested by 7 different restriction enzymes (Hin f1, Hae III, Eco R1, Rsa 1, TAQ 1 Hind III and Bam H1), separted by agarose gel electrophoresis and transferred to nitrocellulose membranes using Southern blotting. Two different radiolabelled probes (M13 and J14 (human collagen probe)) were hybridized to some of the blots resulting in autoradiographs with various polymorphisms. A cosmid sheep DNA library has been constructed using DNA samples from 10 directly unrelated Suffolk. The library will be used to screen known probes to identify sheep DNA complements. Probes exhibiting polymorphism will be used to screen the Southern blots.

Publications: 88/01 to 88/12

BUNCH, T.D., RUSSELL, N., MACIULIS, A. and WOODWARD, S. 1988. Spider lamb syndrome in Suffolk sheep. Utah Farmer-Stockman. December.

RUSSELL, N., BUNCH, T.D., MACIULIS, A. and WOODWARD, S. 1988. Spider lamb syndrome in Suffolk sheep. American Suffolk Sheep Newsletter. December.

33.013* CRIS0096211
A POLYVALENT VACCINIA VIRUS RECOMBINANT VACCINE FOR BLUETONGUE

BREEZE R G; GORHAM J R; College of Vet Medicine; Washington State University, Pullman, **WASHINGTON** 99164.
Proj. No.: WNV-2-2608
Project Type: SPECIAL GRANT
Agency ID: CSRS Period: 01 SEP 85 to 31 AUG 87

Objectives: The objective is to make progress toward the development of a polyvalent vaccine for bluetongue through cloning the gene for the serotype specific protein of BTV-13 and then expressing it in an infectious vaccinia virus recombinant.

Approach: The approach includes: Identification of the gene segment which codes for the serotype specific protein which induces protective immunity for BTV-13; cloning of the gene for this serotype specific protein; construction of an infectious vaccinia virus vector which expresses the immunogenic protein of BTV-13; and demonstration of humoral and cellular immunity and protection against live BTV-13 challenge in mice and sheep vaccinated with this vaccinia vector.

Progress: 86/01 to 86/12. We have made monoclonal antibodies to blue tongue virus serotype 13, and these are presently being characterized. We have also isolated the RNAs of the 10 segments of the virus. Cloning work has not been intiated.

Publications: 86/01 to 86/12
NO PUBLICATIONS REPORTED THIS PERIOD.

33.014 CRIS0132911
CHARACTERIZATION OF SCRAPIE AGENT NUCLEIC ACID

MARSH R F; Veterinary Science; University of Wisconsin, Madison, **WISCONSIN** 53706.
Proj. No.: WIS03173 Project Type: CRGO
Agency ID: CRGO Period: 01 SEP 87 to 31 AUG 90

Objectives: Proj. 8701643. The principal objectives of this proposal are to identify and characterize a scrapie-specific nucleic acid. Screening of over 7000 recombinants from c-DNA libraries constructed from nucleic acids extracted from purified preparations from scrapie-infected hamster brain have identified nine scrapie preferential clones by differential hybridization. Four of these inserts have been sequenced, and each has been found to be homologous to mitochondrial nucleic acids. Because of these results, and studies showing that mitochondria have high infectivity, we will concentrate our search on this organelle.

Approach: The principal approaches will be to examine mitochondrial nucleic acids for unintegrated scrapie-specific sequences by gel electrophoresis of radiolabeled samples. Scrapie-induced mitochondrial genomic heterogeneity will be examined by restriction enzyme fragment length polymorphism and S1 analysis using cloned mitochondrial sequences and synthetic oligonucleotides.

Progress: 88/01 to 88/12. Two cDNA libraries were constructed from brain membrane and cytoskeletal preparations purified from scrapie-infected hamster brains. Four recombinants strongly preferential to the scrapie cytoskeletal preparation were identified by the differential hybridization of 7,000 recombinants. These clones were not, however, preferential to total nucleic acids extractaed from scrapie-infected hamster brains. DNA sequence analysis revealed all four clones to have significant sequence similarities to the mouse mitochondrial genome. This correlation led us to consider a mitochondrial association with scrapie infectivity. Brain mitochondria were purified by sucrose gradient density centrifugation and found to contain high infectivity. Removal of mitochondrial outer membranes by osmotic shock or digitonin treatment resulted in no detectable loss of titer.

Publications: 88/01 to 88/12
AIKEN, J.M., WILLIAMSON, J.L. and MARSH, R.F. Evidence of mitochondrial involvement in scrapie infection. J. Virology, In press.

33.015 0141672
CULICOIDES VARIIPENNIS; VECTOR BIOLOGY AND VECTOR COMPETENCE FOR BLUETONGUE VIRUS

WALTON T E; NUNAMAKER R A; HOLBROOK F R; Agricultural Research Service, Laramie, **WYOMING** 82070.
Proj. No.: 5410-34350-001-00D
Project Type: INHOUSE
Agency ID: ARS Period: 01 NOV 86 to 01 NOV 91

Objectives: Investigate variability and systematic status of C. variipennis (CV). Improve procedures for colonization and maintenance of biting midges. Investigate vector-virus-host interactions in BTV cycle. Improve sampling techniques for arthropod vectors. Evaluate seasonal dynamics of the vector-BTV relationship and produce a predictive model. Characterize genetics of CV competence for BTV.

Approach: Electrophoretic, chromatographic (isozyme/hydrocarbon) and EM studies of CVpopulations will be correlated to BTV vector potential. Specific survey techniques will be utilized to evaluate vector-BTV events, correlate with meteorological events and develop a BTV predictive model. Evaluations of vector-virus survey techniques will continue in Wyoming, Colorado and Nebraska. Biologically secure rearing and handling systems for genetically defined CV colonies will be evaluated and a standard manual of techniques prepared. Selected genetic lines of CV will be assayed for oral suscepti- bility to BTV and phenotypic analyses developed using traditional and molecular biologic techniques. Selected lines will be genotypically analyzed for vector competence, biochemical characteristics and morphological characters. Environmental influences on genetic characteris- tics will be lab evaluated.

Progress: 88/01 to 88/12. Cv is the primary US vector of BTV. Wild & colonized adults were marked with RbCl to study flight range. Two natural, genetically distinct US Cv populations were identified isozymically: Cv variipennis in the northeast & Cv sonorensis in the west. Natural Cvs populations show seasonal genetic changes. Established isofemale Cvs lines of known family groups to characterize by infection rates & competence. Cloned Cvs DNA fragments to study genetic diversity. High incubation temperature (26C vs 20C) increased Cvs mortality. Infection with 3 different BTV serotypes caused no differences in Cvs mortality. Examined adult Cv midguts using immunogold-labelled ultrathin cryosections (IGLUC) after infection with BTV-11; BTV was seen on/within erythrocytes & in midgut cells. IGLUC with monoclonal antibody detected BTV in Cv developing oocytes: BTV was not transmitted transovarially to offspring thru 5 gonatrophic cycles, but antigen was detected in yolk bodies & vitelline membrane of oocytes indicating BTV may penetrate the ovarian sheath. Each of the paired Cv salivary glands consists of 5 lobes attached at the gland's base. Glandular cells had abundant endoplasmic reticulum, mitochondria & microtubules; a basal lamina borders the epithelial cells lining the salivary duct. IGLUC detected BTV antigen in cytoplasm & plasma membrane of salivary gland acinar cells & detected mature virions & antigen extracellularly & in cisternae of vacuoles & endoplasmic reticulum.

Publications: 88/01 to 88/12

NUNAMAKER, R.A., WICK, B.C. and NUNAMAKER, C.E. 1988. Salivary glands of female Culicoides variipennis (Diptera:Ceratopogonidae): Morphologic changes associated with maturation and blood-feeding. Proc. International Congr. Entomol. 28:90.

NUNAMAKER, R.A., WICK, B.C. and NUNAMAKER, C.E. 1988. Immunogold labelling of bluetongue virus in cryosections from Culicoides variipennis (Coquillett) salivary gland. Proc. Electron Microscopy Soc. Amer., 372-373.

FRANCIS, B.R., BLANTON, W.E., LITTLEFIELD, J.L. and NUNAMAKER, R.A., Hydrocarbons of the cuticle and hemolymph of the adult honey bee (Apis mellifera Linnaeus). Annals Entom. Soc. Am., Accepted October 25, 1988.

SIEBURTH, P.J. and MARUNIAK, J.E. 1988. Growth characteristics of a cell line from the velvetbean caterpillar, Anticarsia gemmatalis Hubner (Lepidoptera: Noctuidae). In Vitro Cell Devel. Biol. 24:195-198.

SIEBURTH, P.J. and MARUNIAK, J.E. 1988. Susceptibility of a cell line of Anticarsia gemmatalis (Lepidoptera:Noctuidae) to three nuclear polyhedrosis viruses. J. Inverteb. Pathol. 52:453-458.

HOLBROOK, F.R. 1988. Bluetongue in the United States: Status, transmission and control through vector suppression. Bull. Soc. Vector Ecol. 13:350-353.

AKEY, D.H., LUEDKE, A.J. and OSBURN, B.I. 1988. Development of hypersensitivity in cattle to the biting midge (Diptera:Ceratopogonidae). Misc. Publ. Ent. Soc. Amer. 71:22-28.

34.001 CRIS0090227
MECHANISMS OF GENETIC REGULATION DURING CELLULAR DEVELOPMENT

DAVIS F C; Microbiology & Cell Science; University of Florida, Gainesville, **FLORIDA** 32611.
Proj. No.: FLA-MCS-02330 Project Type: STATE
Agency ID: SAES Period: 23 MAY 83 to 31 DEC 88

Objectives: To prepare molecular probes to study gene regulation in early animal development. To characterize the transcription, translation and storage of messenger RNA sequences during oogenesis. To examine the mechanisms for storage of sequestered mRNA sequences in immature oocytes.

Approach: Radio-labeled DNA probes that are complementary to specific messenger RNA sequences from developmentally regulated genes, either complementary DNA or genomic clones, will be prepared. The probes will be used to study the synthesis, processing and utilization of these messenger RNAs during oogenesis and early embryogenesis in order to establish the mechanisms by which regulation of early development occurs.

Progress: 87/10 to 88/09. A H1 gene probe which was previously prepared and contained only the central coding region of a sea urchin H1 histone gene was evaluated to determine if it could be used as a probe for cloning the Urechis caupo H1 histone gene. The probe was shown to hybridize to a mRNA with the molecular size expected for a Urechis H1 histone mRNA and to an approximately 4 kb restriction fragment in Xba I digests of Urechis genomic DNA. The probe proved to not be useful in probing a genomic library because it bound nonspecifically to lambda DNA and no conditions were found to discriminate between the nonspecific binding to lambda DNA and binding to the Xba I restriction fragment.

Publications: 87/10 to 88/09
INGHAM, L.D. and DAVIS, F.C. 1988. Cloning and Characterization of a Core Histone Gene Tandem Repeat in Urechis caupo. Molec. Cell. Biol. 8:4425-4432.

34.002 CRIS0131695
MODEL OF SEX-LINKED SEVERE COMBINED IMMUNODEFICIENCY

FELSBURG P J; SOMBERG R L; WUNDERLI P W; Veterinary Pathobiology; 1301 West Gregory Drive, Urbana, **ILLINOIS** 61801.
Proj. No.: ILLU-70-0984
Project Type: ANIMAL HEALTH
Agency ID: CSRS Period: 15 MAY 87 to 30 SEP 87

Objectives: To develop a breeding colony of female carriers of an X-linked disease. To develop a genetic screening rest to detect clinically normal female carriers of X-linked diseases. To identify and isolate the T cell gene on the X chromosome which is responsible for the SCID mutation.

Approach: Breeding of current carriers. To develop a genetic screening test using restriction fragment length polymorphisms with X-linked DNA probes and look for X chromosome inactivation. Develop a X-linked T cell specific cDNA library to probe SCID T cells for the presence and/or function of these genes.

Progress: 88/01 to 88/12. 1. Three X-chromosome specific DNA probes have been characterized for identification of the canine X-chromosome These include probes for the hypoxanthine phosphoribosyltransferase (HPRT) gene, phosphoglycerate kinase (PGK) gene, and the Duchenne muscular dystrophy (DMD) gene. Two restriction fragment length polymorphisms (RFLPs), a Pst and an EcoR1 RFLP, have been identified for the DMD gene in the dog. Current work is being conducted to identify canine RFLPs for the HPRT and PGK gene in the dog. 2. Our breeding stock of X-linked severe combined immunodeficiency (XSCID) have been tested for DMD polymorphism. Four of our carrier females are heterozygous for the Pst polymorphism). As other X-chromosome polymorphisms are identified, the genomic DNA for our breeding stock will be evaluated for these as well. 3. Somatic cell hybrids, Chinese hamster x canine, have been generated from T lymphocytes for several of our female carriers and normal females to evaluate the pattern of X-chromosome inactivation in the carrier female T lymphocytes vs normal T lymphocytes. 4. Somatic cell hybrids have been generated which carry the canine XSCID X-chromosome. The hybrids will permit the propagation and future cloning of the XSCID X-chromosome.

Publications: 88/01 to 88/12
SOMBERG, R.L., and FELSBURG, P.J. (1988). Chinese hamster x canine, somatic cell hybrid containing the canine SCID X-chromosome, FASEB J.2:470.

34.003 CRIS0137984
CHARACTERIZATION OF ANTIGENIC AND BIOLOGICAL VARIANTS OF EQUINE INFECTIOUS ANEMIA VIRUS

CARPENTER S; Veterinary Microbiology & Preventive Medicine; Iowa State University, Ames, **IOWA** 50011.
Proj. No.: IOWV-480-23-16 Project Type: STATE
Agency ID: CSVM Period: 01 JUL 88 to 30 JUN 92

Objectives: The overall goal of this research project is to structurally characterize antigenic and biological variants of equine infectious anemia virus. These studies will provide a basis from which we can begin to functionally map the lentivirus genome, with the eventual possibility of developing strategies for intervention in viral replication or viral gene expression.

Approach: Initial in vivo studies will be done to further characterize the virulent of variants we previously defined on the basis of in vitro host cell tropism. All variant isolates will be moleculary cloned and the viral env & LTR sequences will be determined. Functional differences in the LTRs will be assessed using chloramphenicol

acetyltransferase (CAT) as the reporter gene.

34.004 CRIS0096216
ANTIGENIC CHARACTERIZATION OF THE MAJOR GLYCOPROTEINS OF EQUINE HERPESVIRUS-I

ALLEN G P; Veterinary Science; University of Kentucky, Lexington, **KENTUCKY** 40506.
Proj. No.: KY01228 Project Type: SPECIAL GRANT
Agency ID: CSRS Period: 01 SEP 85 to 31 AUG 88

Objectives: The objectives of the investigations proposed are to define the complete antigenic structure of the 2 major glycoprotein antigens of equine herpesvirus 1 (EHV-1) and to determine the protective capacity and extent of structural variability among different viral isolates of the defined antigenic determinants.

Approach: To generate a panel of monoclonal antibodies to EHV-1 GP 13 and 14; to use monoclonal antibodies to enumerate, map and functionally characterize the epitopes of the GP's; to determine by DNA sequencing the structural organization of the nucleotide sequences encoding GP 13 and 14; to analyze the extent antigenic and genetic variation within GP 13 and 14 epitopes from different isolates of the virus and to chemically sythesize the peptides putatively demonstrated to contain the amino acid sequences comprising the epitopes of VP13 and 14, to test the ability of individual peptides to react with epitope defined monoclonal antibodies and to stimulate a T-cell proliferative response in EHV-1 primed lymphocytes from horses.

Progress: 88/01 to 88/12. The objective of this project was to identify and characterize the antigens of equine herpesvirus-1 (EHV-1) that stimulate antibody production by the horse. Experimental methods used included western immunoblotting, monoclonal antibody analysis, recombinant DNA techniques, and DNA sequencing. Significant results achieved during the project period included: (a) identification of 6 major glycoprotein antigens on the surface of EHV-1; (b) genomic mapping of the coding sequences for all 6 EHV-1 glycoproteins ; (c) determination of the complete nucleotide sequence and deduced amino acid sequence of the major neutralizing glycoprotein antigen (gp13) of EHV-1; (d) identification of EHV-1 glycoproteins 10, 13, and 14 as targets for the bulk of the equine antibody response to EHV-1 infection; and (e) identification and preliminary characterization of 5 antigenic domains on EHV-1 gp13. These results should provide information essential for development of more effective vaccines for EHV-1 disease of the horse.

Publications: 88/01 to 88/12

YEARGAN, M.R., ALLEN, G.P., and BRYANS, J.T. (1985). Rapid subtyping of equine herpesvirus 1 with monoclonal antibodies. J. Clin. Microbiol. 21:694-697.

ALLEN, G.P. and YEARGAN, M.R. (1987). Use of lambda-gt11 and monoclonal antibodies to map the genes for the six major glycoproteins of equine herpesvirus 1. J. Virology 61:2454-2461.

ALLEN, G.P. and COOGLE, L.D. (1988). Characterization of an equine herpesvirus type 1 gene encoding a glycoprotein (gp13) with homology to herpes simplex virus glycoprotein C. J. Virology 62:2850-2858.

ALLEN, G.P., YEARGAN, M.R., and COOGLE, L.D. (1988). Equid herpesvirus-1 glycoprotein 13 (gp13): epitope analysis, gene structure, and expression in E. coli. Equine Infectious Diseases (in press).

34.005 CRIS0080968
IDENTIFICATION AND DEFINITION OF THE EQUINE MAJOR HISTOCOMPATIBILITY COMPLEX

BAILEY E F; Veterinary Science; University of Kentucky, Lexington, **KENTUCKY** 40506.
Proj. No.: KY00392 Project Type: ANIMAL HEALTH
Agency ID: CSRS Period: 01 JAN 87 to 31 DEC 91

Objectives: The objectives of this proposal are to investigate physiological traits encoded by the equine major histocompatibility complex (MHC) and influencing disease susceptibility and reproduction.

Approach: The approach to these objectives will be to identify genetic markers of the equine major histocompatibility complex using serology of lymphocyte alloantigens and Southern blotting; map the location of the genes relative to each other; identify physiological effects of the MHC by comparing the performance of horses with different MHC genes for aspects of reproduction and disease susceptibility.

Progress: 88/01 to 88/12. DNA probes from human and mouse class I and class II genes cross-hybridized readily with restriction enzyme digested equine DNA to produce restriction fragment length polymorphisms (RFLP). Comparison of the RFLP markers to serological markers for the equine lymphocyte antigen (ELA) system demonstrated that the two methods identify genes in the same system. RFLP markers revealed more polymorphism than serology and distinguished different haplo-types for horses which are homozygous for serologically identified alleles. Susceptibility to sarcoid tumors and segregation distortion are two physiological traits associated with serological markers of the ELA system. Southern blot analysis was used to investigate RFLPs which might be more strongly associated with the traits. The murine class II probe pAAC6 for A alpha gene was hybridized with a blot of PVU II digested DNA from 10 horses which had sarcoid tumors. No evidence was found for a class II haplotype common to all horses. The murine pB1.4 probe for the murine TCP-1B gene was hybridized to PVU II digested DNA from 14 horses with different ELA types. All horses shared a common restriction fragment.

Publications: 88/01 to 88/12

BAILEY, E., J.G. WOODWARD, D.G. ALBRIGHT, A.J. ALEXANDER (1988) Use of RFLP marker genes to study serological and physiological traits of the horse. IN: Molecular Biol of the Major Histocompatibility Complex of Domestic Animal Species.

BAILEY, E., H. S. CONBOY, P. McCARTHY (1988) Neonatal isoerythrolysis of foals: an update on testing. IN: (FJ Milne, ed) Proc 23rd Ann Conv Am Assoc Equine Practitioners. p 341-353.

BAILEY, E., D. ALBRIGHT, P.J. HENNEY (1988) Equine neonatal isoerythrolysis; evidence for prevention by maternal anti-Ca antibodies. Am. J. Vet. Res 49:1218-1222.

MAC CLUER, J.W., E. BAILEY, L.R. WEITKAMP, J. BLANGERO (1988) ELA and fertility in American Standardbred horses. Animal Genetics (in press).

LAZARY, S., D. F. ANTCZAK, E. BAILEY, et al (1988) Joint report of 5th Intnl Workshop on Lymphocyte Alloantigens of the Horse, Baton Rouge, LA. Animal Genetics (in press).

34.006 CRIS0096827

EIAV PROVIRUS: STRUCTURE, EXPRESSION, AND DIAGNOSTIC POTENTIAL

MONTELARO R C; Biochemistry; Louisiana State University & A&M Col, Baton Rouge, **LOUISIANA** 70803.

Proj. No.: LA-8502128 Project Type: CRGO
Agency ID: CRGO Period: 15 SEP 85 to 30 JUN 88

Objectives: Proj 8502128. The goals of this research program are to examine in detail the transfer, expression, and regulation of equine infectious anemia virus (EIAV) genes during a persistent infection of horses. The results of these studies will then be used to evaluate the potential of nucleic acid hybridization as a diagnostic procedure for EIAV infections.

Approach: The general experimental approach will be to clone and characterize by restriction enzyme mapping the DNA provirus of a cell-adapted strain of EIAV. This provirus clone will then be used as a virus specific probe in hybridization experiments to determine the host tissues infected by virus, the sites of provirus integration and copy number, and the level of virus specific transcription during various stages of disease in experimentally and naturally infected animals.

Progress: 85/09 to 88/06. The primary focus of this research program has been to examine the flow of viral genetic information during persistent and cytopathic infections of cultured cells by EIAV and in leukocytes of infected horses during various stages of disease. As a foundation for these molecular biology studies, a full length proviral clone of EIAV was isolated and its complete nucleotide sequence was determined. Using a combination of specific proviral subclones and synthetic oligonucleotides as probes, the structure and integration patterns of EIAV proviral DNA and patterns of viral transcription were examined in persistent and cytopathic infections of cultured cells. The results of these studies revealed distinct patterns of proviral integration and viral mRNA's in persistent and cytopathic infections. Moreover, cytopathology by EIAV appeared to be correlated with high levels of proviral integration and a relative abundance of spliced viral mRNA. Limited viral nucleic acid analyses of leukocytes isolated from experimentally infected horses during acute stages of disease revealed proviral DNA and viral mRNA patterns similar to those observed in cytopathic infections of cultured cells. In contrast, viral nucleic acids could not be detected by standard hybridization techniques in leukocytes isolated from EIAV infected horses during asymptomatic stages of chronic EIA.

Publications: 85/09 to 88/06

RUSHLOW, K., OLSEN, K., STEIGLER, G., PAYNE, S., MONTELARO, R. and ISSEL, C. 1986. Lentivirus genomic organization: The complete nucleotide sequence on the env gene region of EIAV. Virology 155: 309-321.

RASTY, S., DHRUVA, B., SHILTZ, R., SHIH, D., ISSEL, C. and MONTELARO, R. 1988. Proviral DNA integration and transcriptional patterns of EIAV during persistent and cytopathic infections. J. Virology, in press.

34.007 CRIS0130341

MOLECULAR STUDIES FOR DEVELOPMENT OF AN EQUINE HERPESVIRUS VACCINE

O'CALLAGHAN D; Louisiana State University, Shreveport, **LOUISIANA** 71130.

Proj. No.: LAR-8601204 Project Type: CRGO
Agency ID: CRGO Period: 15 SEP 86 to 30 SEP 89

Objectives: PROJECT 8601204. To identify the genes of equine herpesvirus type 1 (equine abortion virus) that encode viral proteins bearing epitopes capable of initiating a protective immune response.

Approach: To employ the biotechnologies of monoclonal antibody production and cloning of viral DNA fragments in expression vector systems. Monoclonal antibodies specific for EHV-1 proteins will be prepared and assayed for virus neutralizing capacity. These antibodies will be employed to identify viral proteins produced in bacterial cells or animal cells containing EHV-1 DNA sequences introduced as a recombinant DNA molecule within an expression vector. The alternative approach will concern development of recombinant vaccinia virus that would contain and express EHV-1 DNA sequences in cells infected with the (EHV-1 vaccina) virus. Additional studies will concern the identification and characterization of the viral neutralizing polypeptide(s) as well as the viral genes and messenger RNA species encoding these proteins. An understanding of the nature of viral genes encoding proteins important in the immune response is important in the development of strategies to prepare a vaccine for this animal pathogen.

Progress: 86/09 to 89/09. With regard to the development of an equine herpesvirus type 1 (EHV-1) vaccine, we have begun a detailed characterization of two of the major EHV-1 glycoproteins (gp): gp14 (m.u. 0.403-0.413), the EHV-1 equivalent of herpes simplex virus (HSV) gB, and gp17/18 (m.u. 0.841-0.874), the EHV-1 equivalent of HSV gE. The synthesis and processing of gp14 in infected cells have been analysed using a panel of seven monoclonal antibodies (McAb; gift from Dr. George Allen, University of Kentucky). McAb immunoprecipitation of extracts of EHV-1 infected rabbit kidney cells revealed three polypeptides with molecular weights of 138K, 77-75K, and 55-53K. Use of metabolic inhibitors to detect immediate early (cycloheximide/actinomycin D), early (phosphonoacetic acid) and late (no inhibitor) polypeptides indicated that gp14 is synthesized as an early (beta) class molecule. Time course studies showed that the 138K polypeptide is synthesized first (4 hr post infection) while the 77-75K and 55-53K polypeptides appear later (5 hr post infection), suggesting a precursor-product relationship. Pulse-chase experiments confirmed this relationship in that the 138K polypeptide is cleaved into the two smaller forms. Western blot analysis of infected cells extracts subjected to SDS-PAGE in the presence or absence of beta-mercaptoethanol revealed that the 75-77K and 55-53K polypeptides were disulfide bonded.

Publications: 86/09 to 89/09

CAUGHMAN, G.B., ROBERTSON, A.T., GRAY, W.L., SULLIVAN, D.C. and O'CALLAGHAN, D.J. 1988. Characterization of quine herpesvirus type 1 immediate early proteins. Virol. 163: 563-571.

ROBERTSON, A.T., CAUGHMAN, G.B., GRAY, W.L., BAUMANN, R.P. and O'CALLAGHAN, D.J. 1988. Analysis of the in vitro translation products of the immediate early RNA of equine herpesvirus type 1. Virol. In press.

BAUMANN, R.P., YALAMANCHILI, R. and O'CALLAGHAN, D.J. 1988. Functional mapping and DNA sequence of an equine herpesvirus type 1 origin of replication. J. Virol. Submitted.

SULLIVAN, D.C., ALLEN, G. and O'CALLAGHAN, D.J. 1988. Synthesis and processing of glycoprotein 17/18 of equine herpesvirus type 1. 13th International Herpesvirus Workshop. Irvine, CA (162).

CAUGHMAN, G.B. 1988. Equine herpesvirus type 1 persistent infection: Alterations in synthesis and cellular distribution of infected cell polypeptides. 13th International Herpesvirus Workshop. Irvine, CA (145).

BAUMANN, R.P., YALAMANCHILI, R., STACZEK, J. and O'CALLAGHAN, D.J. 1988. Identification of an equine herpesvirus type 1 origin of replication. 13th International Herpesvirus Workshop. Irvine, CA (90).

34.008* CRIS0096291

THE PRODUCTION OF CHIMERIC BOVINE IMMUNOGLOBIN GENES

OSBORNE B A; Veterinary & Animal Science; University of Massachusetts, Amherst, **MASSACHUSETTS** 01003.

Proj. No.: MAS00601 Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 85 to 30 SEP 90

Objectives: The availability of monoclonal antibodies has made immunotherapy feasible for disease control and prevention. However, repeated administration of a non-host immunoglobulin can result in hypersensitivity reactions or neutralization of the "foreign" molecule. Host immunoglobulin would be ideal for immunotherapy, since it would function better with the host's effector system and be less immunogenic. But in animals of veterinary interest, the desired antigen specificities are difficult to obtain. In murine systems, however, almost any desired antigen specificity is easy to obtain. A chimeric immunoglobulin molecule consisting of a bovine constant region and a murine variable region would match the effector functions of the molecule to the host and provide the virtually limitless specificities obtainable from the murine system.

Approach: The approach will include cloning bovine immunoglobulin genes, determining their DNA sequence and utilizing cloned murine variable region genes of known antigen binding specificity to create chimeric bovine-murine immunoglobulin molecules possessing the antigen specificity of the murine variable region and having the effector function of the bovine gene.

Progress: 87/10 to 88/09. During the past year we have isolated a number of immunoglobulin genes from a bovine spleen cDNA library. We have isolated a full length mu cDNA and six lambda light chain genes. We also have isolated two genomic mu clones. The DNA sequence of these clones has been determined. Additionally, these clones have been used for probes in Southern blot analysis. A number of interesting findings have resulted from this work. We have shown that unlike the mouse, human, rabbit and rat, the cow appears to possess only one or at most two C lambda genes. All six of our lambda cDNA clones have an identical sequence indicating they represent the same gene. Southern blot analysis also indicate that the C lambda gene is most probably a single copy gene. The bovine C mu is quite similar to its counterpart in mouse and man except that it appears to be 2-300 bp longer than either the human or mouse sequence. It is not clear at present whether these extra sequences constitute a new domain or whether the CH3 or CH4 domain is twice as long as the mouse or human CH3 or CH4 domains. Further sequencing is being done to answer this question. One manuscript is in preparation on the DNA sequences of both the C lambda and the C mu genes. Lastly, we are in the process of isolating the bovine C kappa gene. We intend to use the lambda and the kappa genes in studies to determine the order of rearrangement of bovine light chains.

Publications: 87/10 to 88/09
NO PUBLICATIONS REPORTED THIS PERIOD.

34.009 CRISO134966
RESTRICTION FRAGMENT LENGTH POLYMORPHISM OF THE VON WILLEBRAND FACTOR GENE IN NORMAL DOGS AND DOGS

RILEY L; JOHNSON G; College of Vet Medicine; University of Missouri, Columbia, **MISSOURI** 65211.
Proj. No.: MO.V-4-30 Project Type: STATE
Agency ID: CSVM Period: 01 JUL 88 to 31 JUN 89

Objectives: Use DNA restriction fragment length polymorphism (RFLP) to study the structure of the gene for von Willebrand factor (vWf) in normal dogs and in dogs with various types and subtypes of von Willebrands disease (vWd). Information obtained should allow us to evaluate the feasibility of using RFLP analysis in genotypic assays to diagnose canine vWd and to differentiate subtypes of the disease.

Approach: Label cDNA for human vWf with the radio-isotope, P; isolate genomic DNA from the leukocytes in blood from normal dogs and from dogs with vWd; optimize conditions for hybridization of genomic canine DNA for vWf and the corresponding human cDNA; determine RFLP patterns for normal canine genomic DNA for vWf; determine RFLP patterns for genomic DNA for vWf from representative dogs with various subtypes of canine vWd.

Progress: 88/07 to 88/12. The most common inherited bleeding disease of dogs is von Willebrand's disease (vWd), a bleeding diathesis caused by a deficiency of von Willebrand's factor (vWf). Current procedures for measurement of vWf and subsequent diagnosis of the disease are unreliable. The goal of this project is to determine the efficacy of restriction fragment linked polymorphisms (RFLPs) in diagnosis of the disease in canines. To date, we have established techniques in our laboratory for isolation of high molecular weight genomic DNA from fresh as well as frozen canine blood leukocytes. We have also acquired four plasmids from Dr. J.E. Sadler which contain the entire structural gene for vWf and include portions of the 5' and 3' flanking regions. DNA fragments which code for vWf have been purified from plasmid sequences by cleaving with restriction endonucleases, agarose gel electrophoresis and electroelution of bands containing the appropriate sequences. Optimal conditions for detection of RFLPs in canine DNA using non-radioactively labeled DNA probes in Southern blot analysis have been established by comparing results with various types of membranes, lengths of DNA transfer, hybridization conditions and wash stringencies. We have also identified conditions which will allow hybridized probes to be removed and membranes hybridized a second time with another labeled cDNA, allowing us to obtain maximum information from a single Southern transfer.

Publications: 88/07 to 88/12
No publications reported this period.

34.010* CRISO135591
CHARACTERIZATION OF SYLVATIC AND DOMESTIC T. SPIRALIS ISOLATES BY DNA PROFILING AND ANTIGEN ANALYSIS

WORLEY D E; BURGESS D E; Veterinary Research Laboratory; Montana State University, Bozeman, **MONTANA** 59717.
Proj. No.: MONB00429Project Type: ANIMAL HEALTH
Agency ID: CSRS Period: 19 AUG 88 to 30 SEP 89

Objectives: To compare strains of Trichinella spiralis from various wild hosts and from domestic pigs using DNA sequence patterns and monoclonal antibodies.

Approach: Strains of Trichinella spiralis obtained from wild carnivores and domestic pigs will be maintained by periodic passage in Swiss Webster or deer mice. Muscle larvae reared in rodents will be collected by peptic digestion, washed in PBS, and used to prepare worm homogenates for electrophoresis and other analytical procedures, including restriction fragment length polymorphism (RFLP) analysis and polyacrylamide gel electrophoresis (SDS-PAGE). Portions of gels will be stained by Coomassie blue and silver nitrate and other portions will be used to prepare western blots. Deer mouse sera will be used to probe these blots.

Progress: 88/01 to 88/12. Six different Trichinella isolates from various sylvatic carnivores, and one isolate from a domestic pig were examined and compared by RFLP (restriction enzyme fragment length polymorphism) patterns of genomic DNA. Restriction enzyme fragments generated by the EcoRI restriction (Fig. 1) show a unique band of repetitive fragments in the Thai Pig isolate at about 1.8 kb which are not present in the sylvatic isolates. Conversely, there are unique bands in the sylvatic isolates at about 5.0 kb which are not present in the Thai Pig isolate. Patterns between 6 sylvatic isolates are nearly identical. Genomic DNA fragments generated by digestion with the Hind III restriction enzyme show unique bands in Thai Pig DNA at 1.5 kb, 1.8 kb, and 6.0 kb which are not present in the sylvatic isolates. A unique band occurs in lynx DNA at 9.8 kb which is not present in any of the other sylvatic isolates. Marked differences were also revealed in the antigens recognized between antigenic sources as well as by sera from mice infected with different strains when T. spiralis muscle larvae were separated by SDS-PAGE, electroblotted and probed with these sera.

Publications: 88/01 to 88/12
WORLEY, D.E., SEESEE, F.M. and ZARLENGA, D.S. 1988. Differential freezing resistance of T. spiralis isolates from MT & Alberta gray wolves w/observations on subspecific relationships of the MT biotype. Proc. Helm. Soc. Wash. sub.

34.011* CRIS0045857
FOOT-AND-MOUTH GENE AND ANTIGENIC STRUCTURE: EXPRESSION OF FMDV IMMUNOGENS

MOORE D M; GRUBMAN M J; KENDALL J;
Agricultural Research Service; Plum Island Animal Dis Center, Orient Point, **NEW YORK** 11944.
Proj. No.: 1940-20460-045-00D
Project Type: INHOUSE
Agency ID: ARS Period: 21 NOV 79 to 30 SEP 86

Objectives: To determine the structure (sequence) of immunogens of FMDV and determine the basis for the immune response to FMDV. To compare the variability of FMDV strains through analysis of the genome products and functions. To study and develop protein vaccines produced by chemical synthesis, through gene expression in procaryotes, eucaryotes, and in infectious virusvectors.

Approach: Identify the polypeptide sequences of virus structural and other viral encoded proteins through nucleotide sequencing of cloned viral genetic material or directly sequence selected polypeptides. Compare sequences of variants to study variation in antigenicity of FMDV. Study the basis of immunization through the preparation of experimental vaccines using poly- peptides generated by chemical synthesis, or biosynthesis in cells engineered to produce viral proteins. Immunize laboratory animals and livestock to determine immunization and protection against FMD with variouspolypeptide vaccines. Identify the location and structure of antigenic sites through competitive inhibition using selected synthetic peptides. Explore the feasibility of constructing native viral antigenic structures as vaccines through expression of viral genes in transformed cells or by viral vectored FMD genes. Study the processing of viral antigens through selective cloning techniques.--Plum Island, NY, Molec. Biol. Lab. 101-C, BL-3, 1/29/80, DM Moore/DO Morgan/MJ Grubman/JL Card/M Zellner/KH Axelson.

Progress: 86/01 to 86/12. Gene segments coding for FMDV viral protein VP-1 cloned into bacterial plasmid expression vectors synthesize high levels of the polypeptide in E. coli. Type A12 VP-1 has been extensively tested in cattle to evaluate the effectiveness of the protein vaccine. High levels of immunity were obtained in the majority of animals and reduced severity of symptoms was observed for animals which became infected on challenge of immunity. Virus type 01 VP-1 has previously generated poor immunity in livestock. Recent tests with a combined polypeptide of two areas of the VP-1 protein generated moderate protective levels in cattle vaccinated twice. In connection with antigenic analysis of VP-1 of type A and O FMDV, variants resistant to specific neutralizing monoclonal antibodies were generated. The variants are analyzed for nucleotide sequence changes in the RNA genome to pinpoint the important sites relating to immunization. The gene segment for VP-1 has been isolated and engineered into vaccinia virus as an experimental, live recombinant viral vaccine. Cell cultures infected with the recombinant virus synthesize FMDV VP-1, but vaccination of guinea pigs, cattle and mice failed to mount an antiviral response. It is likely that the cytoplasmically located VP-1 antigen was not released or that the immunogenicity of the VP-1 as presented to the host was poor. Experiments are being extended to incorporate additional structural components to facilitate morphogenesis and increase potency.

Publications: 86/01 to 86/12
NO PUBLICATIONS REPORTED THIS PERIOD.

34.012* CRIS0140880
PRIMARY STRUCTURE OF THE FMDV GENOME AND GENERATION OF INFECTIOUS DNA CLONES

MOORE D M; WIMMER E; VAKHARIA V; Microbiology; State University of New York, Stony Brook, **NEW YORK** 11794.
Proj. No.: 1940-34000-012-04S
Project Type: COOPERATIVE AGREE.
Agency ID: ARS Period: 01 OCT 85 to 30 SEP 87

Objectives: To develop rapid methods to sequence and study the primary structure of theFMDV genome to determine function of the genome and antigenic characteristics of capsid protein antigens. To examine the transcription and translation in vitro and the expression of cDNA segments of FMDV protein coding regions in tissue cultures. To study the processing of FMDV polyproteins and evaluate the possibility of generating native structures of the capsid proteins of the virus.

Approach: Sequence data will be obtained from the genomic RNA or cloned cDNA segmentsof the FMDV genome. Specific areas of the genome will be selected for study, with the non-coding areas of the genome, the capsid protein coding regions, and coding regions for other non-structural proteins. Specialized vectors containing protein coding cDNA segments of the FMDV genome will be transcribed in vitro and subsequently translated in vitro. Vectors will be transfected into mammalian tissue culture cells to study the expression/ assembly of the capsid region. The function of non-structural proteins and the antigenicity of capsid structural proteins will be studied in this manner. The regeneration of progeny virus from cloned cDNA will be considered by transfection of either plasmids containing full-length genomeinserts or transfection of RNA transcripts of cDNA clones.

Progress: 88/01 to 88/12. Previously, in vitro transcription/translation systems were used to determine the requirements for expression and proteolytic processing of the capsid polyprotein precursor molecule into the individual capsid proteins. To examine the processing of FMDV proteins expressed in vivo, two transient expression systems were developed. Different segments of the coding sequence of the FMDV genome were cloned into plasmids containing either the bacteriophage T7 promoter or a vaccinia late promoter. These were transfected into tissue culture cells infected with a recombinant vaccinia virus expressing the T7 RNA polymerase or with wild

type vaccinia virus WR, respectively. Cells were harvested and extracts of the cells were analyzed by western blot analysis with a VP1 antiserum/125I-protein A detection system. The results showed that the P1-2A region was efficiently cleaved from precursor polyprotein and that the P1 region was further processed to capsid polypeptides if the clones contained the coding sequence for the viral protease, 3C. The results indicate that FMDV proteins expressed under the control of the T7 promoter or a vaccinia promoter can be effectively processed into capsid proteins in vivo and that stable vaccinia virus recombinants should be able to be engineered to express the same proteins. Work is underway to engineer and isolate such recombinant vaccinia viruses.

Publications: 88/01 to 88/12

VAKHARIA, V.N., DEVANEY, M.A., GRUBMAN, M.J., and MOORE, D.M. 1988. Cloning and expression of foot-and-mouth disease virus genes. XI Pan American Congress of Veterinary Sciences. Lima, Peru. (Abstract).

34.013* CRIS0095550
ATTENUATED SALMONELLA VACCINE

BENSON C; Clinical Studies; New Bolton Center, Kennett Square, **PENNSYLVANIA** 19348.
Proj. No.: PENV-5-20383 Project Type: STATE
Agency ID: CSVM Period: 15 DEC 83 to 30 SEP 88

Objectives: The generation of attenuated strains of Salmonella which may be utilized to immunize horses and food animals by the oral route.

Approach: Several isolates from clinical cases of Salmonellosis will be attenuated by selective transposon insertion and deletion (Bochner technique). Attenuation will be assessed by an in vitro procedure and a mouse protection:challenge system prior to trial immunization in the equine and bovine species. The presence of an enterotoxin gene in Salmonella will be determined by gene hybridization analysis utilizing several different genetic probes. The enterotoxin gene will be isolated and the nucleotide sequence varied to produce a toxoid. The attenuated gene will be inserted into the genome of vaccine strains by transposon insertion.

Progress: 88/01 to 88/12. An attenuated vaccine of Salmonella group B has been developed and tested extensively in mice. One dose (10 cfu/dose) administered per os to Balb/c mice was sufficient to protect against a 10 MLD of a virulent isogenic strain. Two vaccine doses, per os at 2 week intervals, stimulated a sufficient level of cross-immunity to protect against a 10 MLD dose of a highly virulent strain of S. enteritidis. This vaccine is intended for use in bovine, equine and avian species.

Publications: 88/01 to 88/12

NO PUBLICATIONS REPORTED THIS PERIOD.

34.014 CRIS0133834
INDUCIBLE CYTOCHROMES P-450 FROM HEPATIC MITOCHONDRIA

AVADHANI N G; RAZA H; BHAT K S; Animal Biology; 3800 Spruce Street, Phildelphia, **PENNSYLVANIA** 19104.
Proj. No.: PENV-5-23963 Project Type: STATE
Agency ID: CSVM Period: 05 SEP 85 to 31 AUG 90

Objectives: The objective of this proposal for the coming year is to isolate cDNA clones from the gt11 expression library using highly specific monoclonal antibodies to all three of the mitochondrial specific cytochromes P-450 inducible with 3-methyl cholanthrene and phenobarbital.

Approach: The cDNA clones will be sequenced from both ends of the insert using M(13) plus and minus cloning strategy and dideoxy sequencing method. The sequences coding for the N-terminal signal sequence unique for almost all mitochondria distined proteins, will be derived and the true mitochondrial distination for these purified cytochromes P-450 will be ascertained. Parallel investigation on the chromosomal location of genes coding for the mitochondrial P-450 isoforms will be undertaken. For this purpose, a rat genomic library in EMBL-3 cosmid vector has been constructed. The library will be screened with cDNA clones and the genes will be mapped using restriction mapping, genome walking and in situ hybridization to G banded chromosomes.

34.015 CRIS0133837
CRYSTALLINS IN NORMAL AND REGENERATED NEWT EYE LENSES

MCDEVITT D S; Animal Biology; 3800 Spruce Street, Phildelphia, **PENNSYLVANIA** 19104.
Proj. No.: PENV-5-20918 Project Type: STATE
Agency ID: CSVM Period: 01 SEP 78 to 30 JUN 89

Objectives: It is the objective of this proposal to initiate a detailed study of the structure of the crystallin genes, initially o1A crystallin, in the Eastern Spotted Newt, Notophthalmus viridescens. The adult newt is unique in its ability to regenerate in vivo a lens from the dorsal iris epithelium after lentectomy.

Approach: To this end, newt cDNA and genomic libraries will be established, and newt o1A crystallin cDNA's isolated. These cDNA's will be used as probes to isolate the newt o1A crystallin gene from the newt genomic DNA library created. Sequencing of o1A crystallin cDNA's and subsequent sequencing of the o1A crystallin gene (it is expected to be single copy), will permit investigation of those upstream and downstream elements of the gene locus considered to be involved in transcription and expression, as well as to establish homology to known crystallin gene structure of other vertebrates. These cDNA's will later also be utilized as probes to establish gene (expressed as mRNA) activity in

embryonic lenses (as a baseline), iris tissue and lens regenerates.

Progress: 87/07 to 88/06. The previous application's specific aims were to continue to establish the physical similarity, localization and patterns of appearance and synthesis of the crystallins, proteins characteristic of the eye lens, in developing, regenerating and fully-regenerated lenses of the salamander Notophthalmus viridescens. It was also an objective of the proposal to begin an investigation of the "factor(s)" (in the classical sense) in the ocular milieu thought to be casual to and/or necessary for the maintenance of regeneration of the eye lens in these amphibians. The P.I. spent a sabbatical (1983-1984) in the laboratory of Dr. Joram Piatigorsky, Chief, Laboratory of Molecular and Developmental Biology, N.E.I., N.I.H. The Study Section that reviewed the last Competitive Continuation of this proposal had recommended that the P.I. seek additional training, in molecular biology, to enable its future application to the newt lens regeneration system. During this period, the P.I. gained hands-on experience and knowledge of most of the modern methodology available for analysis of gene structure and expression. Recombinant DNA techniques were successfully used by the P.I. during this time to isolate and characterize for the first time the human yield alpha a crystallin gene (McDevitt et al., 1984, 1987).

Publications: 87/07 to 88/06

BRAHMA, S.K., MCDEVITT, D.S., and DEFIZE, L.H.K. 1987. Ontogeny of alphaA and alphaB crystallin polypeptides during Rana temporaria lens development. Exp. Eye Res., 45, 253-261.

MCDEVITT, D.S. 1989. In: Transdifferentiation in Animals - A Model for Differentiation Control. "Genomic Adaptability in Cell Specialization of Eukaryotes" (eds. Marie A. DiBerardino and Laurence D. Etkin).

BORST, D.E. and MCDEVITT, D.S. 1987. Eye lens regeneration and the crystallins in the adult newt, Notophthalmus viridescens. Exp. Eye Res. 45, 419-441.

34.016 0097881
BLUETONGUE AND EPIZOOTIC HEMORRHAGIC DISEASES IN RUMINANTS

COLLISSON E W; LIVINGSTON C W; Veterinary Microbiology & Parasitology; Texas A&M University, College Station, **TEXAS** 77843.
Proj. No.: TEX06808 Project Type: HATCH
Agency ID: CSRS Period: 01 FEB 86 to 31 JAN 91

Objectives: To develop recombinant DNA probes for bluetongue (BT) and epizootic hemorrhagic disease (EHD) viruses. To develop techniques for using these probes as a practical diagnostic tool for the clinical laboratory. To use these probes to study the pathogenesis of these viruses in the acutely infected chick embryo, sheep, and acutely and persistently infected cattle. To study the molecular epizoology of BTV and EHDV. To determine the indigenous serotypes of BTV and EHDV in Texas for future control programs. To determine gene assignments of EHDV.

Approach: Recombinant DNA techniques will be used to develop probes for detection of BTV and EHDV genomes in infected tissues. Characterization of viral genomes will be done by such techniques as polyacrylamide gel electrophoresis (PAGE) and oligonucleotide fingerprinting, whereas proteins will be analyzed by HPLC in addition to PAGE. The extent of the diversity of BTV in Texas will be determined by monitoring seroconversion of sentinel cattle and doing retrospective isolation of the viral strains. Genome reassortment procedures, as well as in vitro translation techniques will be used to map EHDV genes.

Progress: 88/01 to 88/12. In situ cytohybridization was used to determine the tissue tropism and target cells for replication of bluetongue virus (BTV) in the developing chicken embryo. Hybridization with a biotinylated probe detected viral replication in embryos inoculated with the four U.S. serotypes and a BTV field strain. At the final stages of infection, when the embryos were hemorrhagic, viral infection could consistently be detected in the brain, kidney, spinal cord, heart, lung and liver with the brain and kidney most severly affected. Other tissues, such as the retina, skin, tongue and intestinal villi also supported viral replication with greater concentration of virus localized with in epithelial cells, such as those lining the kidney tubules and tertiary bronchi of the lungs. Within 24 hours after inoculation, viral replication occurs initially in the brain and kidney. By 48 hours, viral replication can also be detected in the lungs, heart and spinal cord with the liver being severely infected by 72 hours. Low levels of hybridization could be detected in embryos infected with EHDV.

Publications: 88/01 to 88/12

WANG, L., KEMP, M., ROY, P. and COLLISSON, E. 1988. Tissue tropsim and target cells of bluetongue virus in the chicken embryo. J. Virol. 62: 887-893.

COLLISSON, E., WANG, L., ROY, P. and KEMP, M. 1988. Detection of BTV in infected chicken embryos by in situ hybridization in orbiviruses and birnaviruses in the Proc. Double Stranded RNA Virus Symposium.

34.017 CRIS0137535
KERATIN GENOTYPE POLYMORPHISM & PHENOTYPE VARIABILITY IN THE NORMAL & ABNORMAL EQUINE HOOF

HOOD D M; Veterinary Physiology & Pharmacology; Texas A&M University, College Station, **TEXAS** 77843.
Proj. No.: TEX06977 Project Type: ANIMAL HEALTH
Agency ID: CSRS Period: 20 FEB 89 to 30 SEP 91

Objectives: This proposal seeks to establish the data base necessary for mechanistic studies on metabolism and growth of equine hoof. The objectives are to define finite phenotypic markers and index genetic polymorphism critical

to understanding mechanisms of phenotype transformation of keratinizing epithelium. The hypothesis is that visible defects present in the hoof reflect changes occurring at the molecular level and are expressed or mediated by characteristic changes in the keratin subtypes present in the hoof.

Approach: To adequate test this hypothesis it will be necessary to: 1) Acquire keratin containing tissues from five populations of horses documented as being normal or having nutritional deficiencies, inherited defects, hyperplasia of the laminar epithelium, or trauma induced lesions. 2) Isolate and characterize the keratin polypeptide subtypes present using a polyacrylamide gel electrophoresis technique. Comparison of the keratin subtypes present between the partially and fully keratinized tissues, both within and between groups will be made. 3) Index the degree of keratin gene polymorphism (using restriction fragment length polymorphism as a index) existing in the normal horse and compare to the horse with inherited pathologies of the hoof.

34.018 CRIS0090348
RETROVIRUS GENE MAPPING STUDIES

CHEEVERS W P; Microbiology & Pathology; Washington State University, Pullman, **WASHINGTON** 99164.
Proj. No.: WNP00621 Project Type: ANIMAL HEALTH
Agency ID: CSRS Period: 01 MAY 83 to 30 JUN 87

Objectives: To develop gene cloning and mapping strategies for the preparation of specific CAEV cDNA probes for the study of expression and stability of viral genes in CCAEV-induced arthritic lesions in goats.

Approach: To produce an env gene cDNA derived by restriction endonuclease cleavage of a CAEV provirus-pBR 322 recombinant DNA. Methodology will involve molecular cloning and hybrid-arrested cell free translation.

Progress: 87/01 to 87/06. We have finished the physical characterization of a lambda-1059 caprine arthritis-encephalitis (CAE) provirus molecular clone and have begun experiments to analyze CAEV gene expression in cell culture and in clinically affected goats. In addition, we have begun necleotide sequence homology experiments with a lambda-visna provirus clone. Additional clones are being produced with the G-63 and Co strains of CAEV in lambda-EMBL-3.

Publications: 87/01 to 87/06
NO PUBLICATIONS REPORTED THIS PERIOD.

34.019 CRIS0097447
MOLECULAR CLONING OF EQUINE INFECTIOUS ANEMIA PROVIRUS

CHEEVERS W P; Veterinary Microbiology & Pathology; Washington State University, Pullman, **WASHINGTON** 99164.
Proj. No.: WNV-13L-2540-9043Project Type: STATE
Agency ID: CSVM Period: 14 JUN 83 to 31 JUL 84

Objectives: To obtain a recombinant DNA comprised of the entire coding sequence of equine infectious anemia provirus.

Approach: The approach includes: Isolation of closed-circular proviral DNA from infected cells; identification of a unique restriction enzyme cleavage site in coding sequence of proviral DNA; and insertion of proviral DNA into E. coli plasmid pBR 322.

Progress: 83/06 to 84/07. Due to the low amount of provirus recovered from cells infected with equine infectious anemia virus, it was decided to develop the molecular cloning technology with the caprine arthritis-encephalitis retrovirus (CAEV). Unintegrated provirus was purified from virus-infected caprine synovial membrane cells, digested with the restriction enzyme Bam HI and ligated into bacteriophage lambda 1059 DNA. DNA was packaged in vitro and amplified in Escherichia coli Q359. Eleven recombinant phages were selected by plaque hybridization using P-labeled cDNA obtained by reverse transcription of CAEV RNA. Complete CAEV provirus was recovered from one of these clones.

Publications: 83/06 to 84/07
NO PUBLICATIONS REPORTED THIS PERIOD.

34.020 CRIS0097441
SUBUNIT AND SYNTHETIC VACCINES FOR EQUINE INFLUENZA

YILMA T; Veterinary Microbiology & Pathology; Washington State University, Pullman, **WASHINGTON** 99164.
Proj. No.: WNV-10S-3925-0044Project Type: STATE
Agency ID: CSVM Period: 01 JUL 83 to 30 JUN 86

Objectives: The achievement of better and longer-lasting protection against equine influenza through development of a new subunit vaccine and a new synthetic vaccine.

Approach: The approach will include growth of virus, isolation and purification of the haemagglutinin spikes, and subsequent evaluation of the haemagglutinin subunit vaccine in mice. Efforts will be made to establish a reliable model system for testing the subunit vaccine in foals. If the subunit vaccine is found to be effective, synthetic peptide vaccines of varying lengths will be developed and tested.

Progress: 83/07 to 85/12. We have the complete coding sequence of the hemagglutinin and neuraminidase genes for both serotypes of equine influenza virus. Our present objective

is to develop for vaccinia virus recombinants that express the individual HA and NA genes of the two serotypes of equine influenza.

Publications: 83/07 to 85/12
NO PUBLICATIONS REPORTED THIS PERIOD.

34.021* CRIS0138473
RECEPTORS FOR GROWTH HORMONE AND GONADOTROPINS

JI T H; Molecular Biology; University of Wyoming, Laramie, **WYOMING** 82070.
Proj. No.: WYO-247-89 Project Type: HATCH
Agency ID: CSRS Period: 30 JUN 89 to 29 MAY 94

Objectives: Determination of the complete genomic structure of the R(GH) gene and putative GH binding protein gene. The results may reveal the coding sequence of the putative GH binding protein. We would like to extend this study on the human genes to bovine genes. Investigation of the regulatory DNA sequences of these genes. Investigation of expression of these genes during fetal and neonatal development of the rat. Determination of the regulatory elements of hCG genes. Determination of interacting sites of hCG and the receptor.

Approach: DNA will be sequenced as we have routinely done in the past, employing double strands pGEM, sequenase II, alpha- S-ATP, deaza dGTP or dITP, and both the universal and reverse primers. We can routinely determine 350 bases in one direction in one tier system. It is possible to determine the sequence of a 1 kb insert without subcloning. Sequencing gels are read with a gel reader, the data entered into a DNA analysis program and aligned, and global sequence searches through GENBANK. We hope to determine not only the genomic sequences of the two genes but also the coding sequence of the putative GH binding protein.

CM 35 BEES AND HONEY AND OTHER POLLINATING INSECTS

35.001 CRIS0130194
GENETIC CHARACTERIZATION OF HONEYBEES THROUGH DNA ANALYSIS

HALL H G; Entomology & Nematology; University of Florida, Gainesville, **FLORIDA** 32611.
Proj. No.: FLA-ENY-02594 Project Type: CRGO
Agency ID: CRGO Period: 15 JUL 86 to 31 JUL 90

Objectives: PROJ 8600130. To find differences in the DNA of Africanized and European honeybees that can be used for reliable genetic distinction. The results of this work will contribute to basic knowledge of honeybee genetics. The positive identification of the African honeybee by this approach will be valuable for regulatory control measures.

Approach: The approach of the proposal entails the isolation of nuclear DNA from samples of European and African honeybees and from their hybrids. The DNA is digested with a selection of restriction enzymes, the DNA fragments selected by electrophoresis and detected by Southern blotting with radioactive probes. The probes are random fragments of honeybee DNA ccloned as inserts in bacterial plasmids. The major effort of the project involves selecting many probe-restriction enzyme combinations that reveal differences and testing these combinations with many more widespread samples of honeybees.

Progress: 87/10 to 88/09. African honeybees, introduced in Brazil in 1957, have now migrated as far as Mexico. DNA restriction fragment polymorphisms are being found that enable reliable identification of African bees. Furthermore, genetic interactions between African and European honeybee populations can be followed. Nuclear polymorphisms are detected with probes of randomly cloned regions of European bee DNA. To detect mitochondrial DNA, isolated preparations are used as a probe. Multiple samples from the United States, Mexico, Costa Rica, Venezuela, and South Africa have been tested. A most significant finding is that all of 13 randomly caught feral swarms from Mexico, and all samples tested from Costa Rica and Venezulela (total of 14) carry African mtDNA. Because mtDNA is maternally inherited, this clearly demonstrates that African bees have spread as swarms derived from continous maternl lineages from the original introduced bees. Migration has not been through the flight of African drones mating with extant European queens. Established Mexican colonies, carrying European mtDNA, show a loss of European nuclear DNA mrkers and, therefore, have become "Africanized" due to gene flow from drones. However, these hybrids do not contribute to the migrating Africanizing force or persist in established tropical populations. Compared to Venezuelan bees, the feral Mexican swarms carry a greater, although minor, amount of the nuclear European alleles.

Publications: 87/10 to 88/09

HALL, H.G. 1988. Characterization of the African honey-bee genotype by DNA restriction fragments. In Needham, G.R., Page, R.E., Delfinado-Baker, M., Bowman, C.E. eds. Africanized Honey Bees and Bee Mites, Elis Horwood Ltd. pp. 287-293.

HALL, H.G. 1988. Distinguishing African and European honeybees using nuclear DNA restriction fragment polymorphisms. Florida Entomologist 71, 294-299.

HALL, H.G. 1989. Genetic characterization of honey bees through DNA analysis. In Fletcher, D.J.C. and Breed, M.D. eds. The African Honeybee, Westview Press. In press.

35.002 CRIS0138045
IDENTIFICATION, BEHAVIORAL ECOLOGY, GENETICS AND MANAGEMENT OF AFRICAN HONEYBEES

HALL H G; Entomology & Nematology U.s. Horticultural Lab; University of Florida, Gainesville, **FLORIDA** 32611.
Proj. No.: FLA-ENY-02791 Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 88 to 30 SEP 93

Objectives: Develop and improve techniques for identifying individual worker, queen and drone Africanized honeybees (AHBs). Monitor the entry and spread of the AHB genotype into the U.S., and determine contributions of swarming and mating to AHB spread. Before and during African honeybee migration, bee samples will be collected from regions of northern Mexico, Texas, Louisiana, Mississippi, Arizona, California and Florida. The samples will be obtained from trapped drones, feral swarms and established apiaries spatially arranged as transects of grids.

Approach: DNA restriction fragment length polymorphisms (RFLPs) will be sought that can identify African and European honeybees. Samples will be tested for nuclear and mitochondrial DNA RFLPs already characterized and for new nuclear DNA RFLPs expected to be found. Spatial and temporal spread of nuclear and mitochondrial markers will distinguish African bee paternal and maternal gene flow into the extant European populations. Distribution patterns of genotypes within the temperate United States may point to processes affecting hybridization between African and European honeybees.

35.003* CRIS0133059
FLOW CYTOMETRIC DETERMINATION OF INSECT DNA FOR IDENTIFICATION AND CONTROL OF INSECTS

JOHNSON J S; Entomology; Texas A&M University, College Station, **TEXAS** 77843.
Proj. No.: TEX06916 Project Type: HATCH
Agency ID: CSRS Period: 18 SEP 87 to 31 AUG 92

Objectives: 1) Develop flow cytometry and in particular total nuclear DNA content determination as a cytotaxonomic tool, for the recognition of host races, biotypes, and crytic species of insects. 2) Catalogue the nuclear DNA content of insects of economic interest. 3) Develop multiparameter DNA flow cytometry using dyes which are specific for total DNA, adenine and thymine rich repetitive DNA, and guanine and cytosine rich repetitive DNA. 4) Demonstrate the developmental pattern and mode

of inheritance of DNA content variation for both euchromatic (unique) and heterochromatic (repetitive) insect DNA sequences. 5) Develop methodology to sort insect nuclei and chromosomes as an aid to gene cloning in insects.

Approach: Assay the total DNA, and sequence specific DNA content within and between individuals, populations, races, and species of economically important insects as an aid to monitoring insects of economic interest which are subjects of IPM control efforts.

Progress: 88/01 to 88/12. We have now had one year of work with this unique new methodology for the study of heritable variation in insects. We have measured the DNA content of over 1500 individual fire ants including 3 native and two imported species. In addition, we have measured the DNA content of over 200 honey bees, nearly 300 boll weevils, plus smaller number of individuals from 10 other species. The results have been unexpected and exciting. In particular we find: DNA content is a diagnostic tool that aids in identification of species, populations and types. To date, very significant and predictable DNA content differences have been found between 5 fire ants species, between 4 species of boll weevil, and between the Africanized form of the honey bee, the European honey bee and the F-1 hybrid between the two forms. DNA content variation exists within and among some populations. The most extreme variation we have observed occurs in a fire ant population in Walton Co., Georgia, were 129 of 515 males, females, and workers show 3N or triploid DNA amounts. The remainder are diploid or haploid as expected. The smallest significant variation occurs between populations of the boll weevil Anthonomus grandis where DNA content changes are associated with different host plants. DNA change occurs during development. Male fire ants are haploid during early development, but double their DNA before maturity. Boll weevils are largely 2N throughout their life, although in 1/4 to 3/4 of their cells may have 15 to 20 percent additional DNA.

Publications: 88/01 to 88/12

JOHNSTON, J.S., ELLISON, J.R. and VINSON, S.B. 1989. Flow cytometric determination of insect DNA as an aid to the description, identification, and control of the imported fire ant. Vinson SB McCOWN JL (eds.) Proc. Imported Fire Ant Sympo.

JOHNSTON, J.S. and ELLISON, J.R. 1989. DNA heterogeniety within and among fire-ants of the genus Solenopsis in southern United States: Evidence from flow cytometry. Submitted to Cytometry.

JOHNSTON, J.S. and ELLISON, J.R. 1988. Triploidy in a polygynous population of the imported fire ant Solenopsis invicta in Walton Co., Georgia. Submitted to Hereditas.

ELLISON, J.R., VINSON, S.B. and JOHNSTON, J.S. 1989. A method for determination of DNA content of nuclei of insects by flow cytometry. Submitted to Cytometry.

ELLISON, J.R., VINSON, S.B. and JOHNSTON, J.S. 1989. Acquired somatic diploidy in males of the imported fire ant. Submitted to Canadian Jn. Genet. and Cytogenet.

35.004 CRIS0136166
GENETIC AND MOLECULAR APPROACHES TO HONEY BEE IMPROVEMENT

MILNE C P JR; Entomology; Washington State University, Pullman, **WASHINGTON** 99164.
Proj. No.: WNP00818 Project Type: STATE
Agency ID: SAES Period: 01 SEP 88 to 31 AUG 91

Objectives: Develop methods for direct gene transfer into the honey bee. Develop methods to map cloned genes in the honey bee. Conduct short-term research relevant to the Washington beekeeping industry.

Approach: Clone and characterize honey bee transposable elements. Clone and evaluate honey bee genes as candidate reporter genes for transformation. Clone and characterize promoters for tissue, stage and sex specific expression. Evaluate potential beneficial genes for transfer to the honey bee. Perfect the in situ hybridization technique of cloned genes to metaphase chromosomes. Identify specific genomic clones that hybridize to each honey bee chromosome. Develop a bank of chromosome specific genomic clones that saturate the genome. Perfect the Southern mapping technique of cloned genes on honey bee chromosomes. Meet with relevant parties to ascertain acute needs of the Washington beekeeping industry that can be addressed by published research findings or short-term research projects.

35.005 CRIS0136216
MOLECULAR ANALYSES FOR IDENTIFICATION OF AFRICANIZED HONEY BEES (APIS MELLIFERA 1.)

AIKEN J M; Veterinary Science; University of Wisconsin, Madison, **WISCONSIN** 53706.
Proj. No.: WIS03252 Project Type: HATCH - PENDING
Agency ID: CSRS Period: 01 NOV 88 to 30 SEP 92

Objectives: Identify African honey bee DNA sequences that have diagnostic utility for identifying African honey bees.

Approach: Utilize recombinant DNA technology for development of accurate methodology for identification of Africanized honey bees. The proposed research entails: 1) improving AfR3 as a diagnostic probe for identifying African honey bees. 2) identifying potential African specific sequences within AfR3, and 3) isolating and characterizing other diagnostically valuable African honey bee sequences.

38.001* CRIS0089692
STUDY OF THE GENETICS AND GENETIC MODIFICATION OF MICROORGANISMS IMPORTANT IN FOODS

GLATZ B A; HAMMOND E G; PATTEE P A; Food Technology; Iowa State University, Ames, **IOWA** 50011.
Proj. No.: IOW02611 Project Type: STATE
Agency ID: SAES Period: 01 JUL 83 to 30 JUN 87

Objectives: To locate the genes involved in enterotoxin production by Staphylococcus aureus. To determine if plasmids are involved in enterotoxin production in S. aureus. To develop means of controlling enterotoxin production in S. aureus. To devise methods for mutagenizing Propionibacterium strains and other dairy starter cultures. To produce desirable mutants of Propionibacterium. To examine Propionibacterium strains for the presence of plasmids.

Approach: Genetic analysis will be performed by means of conventional mapping procedures such as transformation and transduction as well as by protoplast fusion. Analysis for the presence of plasmids will be by agarose gel electrophoresis of isolated DNA. Mutagenesis will be done by exposure to chemical mutagens and to ultraviolet light.

Progress: 87/07 to 87/12. No progress reported this period.

Publications: 87/07 to 87/12

VORHEES, K.I. and GLATZ, B.A. 1987. Generation, isolation and characterization of auxotrophic mutants of Propionibacterium. J. Dairy Sci. 70 Suppl. 1:79.

PAI, S.L. and GLATZ, B.A. 1987. Production, regeneration and transformation of protoplasts of Propionibacterium strains. J. Dairy Sci. 70 Suppl. 1:80.

REHBERGER, T.G. and GLATZ, B.A. 1987. Characterization of plasmid DNA in Propionibacterium freudenieichii subsp. globosum P93: evidence for plasmid-linked lactose utilization. J. Dairy Sci. 70 Suppl. 1:80.

GLATZ, B.A. 1987. Studies on the genetics of Propionibacteria for their improvement as industrial microorganisms. J. Dairy Sci. 70 Suppl. 1:96.

CM 40 PEOPLE AS WORKERS, CONSUMERS, AND MEMBER OF SOCIETY

40.001 CRIS0089904
MOLECULAR BASIS FOR EXPRESSION OF THE HUMAN ALBUMIN AND ALPHA-FETOPROTEIN GENES

DUGAICZYK A; Biochemistry; University of California, Riverside, **CALIFORNIA** 92521.
Proj. No.: CA-R*-BCH-4333 Project Type: STATE
Agency ID: SAES Period: 01 MAR 83 to 30 SEP 88

Objectives: To understand the molecular basis between structure and expression of the human genes for serum albumin and Alpha-fetoprotein. Since the two genes are evolutionarily related and developmentally regulated, research will focus on their structure and function in man and other species, as well as in normal and in diseases associated with a malfunction of the genes.

Approach: The structure of the genes will be determined by their molecular cloning and subsequent DNA sequence determination. Sequences from different species will be compared for rates of their molecular evolution. Individuals afflicted with malfunctioning of the genes will be screened for differences in their genome within the albumin and Alpha-fetoprotein locus.

Progress: 87/01 to 87/12. We have determined the structure of the human alpha-fetoprotein (AFP) gene. The gene spans 19,489 bp from the putative "Cap" site to the polyA site. It is composed of 15 exons separated by 14 introns, which are symmetrically placed within the three domains of AFP. There are six polymorphic sites within 4,690 base pairs of contiguous DNA derived from two allelic AFP genes. This amounts to a polymorphic frequency of 0.13%, which is about 5-10 times lower than values estimated from studies on polymorphic restriction sites in other regions of the human genome. There are four types of repetitive sequence elements in the introns and flanking regions of the human AFP gene. At least one of these is apparently a novel structure (designated Xba) and is found as a pair of direct repeats. It is conceivable that within the last 2 million years the copy in intron 9 gave rise to the repeat in intron 7. Their present location on both sides of exon 8 gives these sequences a potential for disrupting the functional integrity of the gene in the event of an unequal crossover between them. There are three Alu elements, one of which is in intron 4; the others are located in the 3' flanking region. A solitary Kpn repeat is found in intron e. Neither the Xba nor the Kpn elements are present in the related human albumin gene, whereas Alu's are present in different positions. From phylogenetic evidence, it appears that Alu elements were inserted into the AFP gene at some time postdating the mammalian radiation 85 million years ago.

Publications: 87/01 to 87/12
GIBBS, P.E.M., ZIELINSKI, R., BOYD, C. and DUGAICZYK, A. 1987. Structure, polymorphism, and novel repeated DNA elements revealed by a complete sequence of the human alpha-fetoprotein gene. Biochemistry 26:1332-1434.

GIBBS, P.E.M. and DUGAICZYK, A. 1987. Origin of structural domains of the serum-albumin gene family and a predicted structure of the gene for vitamin D-binding protein. Mol. Biol. Evol. 4:364-379.

40.002 CRIS0047488
GENETICS OF ANOPHELES MOSQUITOES

SEAWRIGHT J A; NARANG S; Agricultural Research Service; University of Florida, Gainesville, **FLORIDA** 32611.
Proj. No.: 6615-20851-020-01S
Project Type: COOPERATIVE AGREE.
Agency ID: ARS Period: 04 DEC 81 to 03 DEC 85

Objectives: Develop electrophoretic techniques to analyze the genomes of laboratory and field populations of Anopheles species..

Approach: Electrophoresis will be used to identify isozymes in Anopheles sp. Linkage relationships will be established for 30-40 loci and a linkage map will be constructed for An. albimanus. Standard, homozygous strains will be established and used in the analysis of field populations..

Progress: 81/12 to 85/12. This is the final report for this cooperative agreement, the primary objectives of which were the assignment of allozyme loci on genetic maps and the use of allozyme analysis for the delineation of the genetic structure of field populations of anopheline mosquitoes. Genetic crosses were used to map the loci for aconitase-2 (Acon-2) and peptidase-4 (pep-4) on chromosomal arms 3L and 2R, respectively, in Anopheles albimanus. Peptidase-1, which is active only in larvae, was assigned to a non-specific position on chromosome 3. The genetic map of A. albimanus now consists of 37 loci, including 12 encyme loci. However, these loci are not evenly distributed, since 7 enzyme loci have been assigned on the gene map of 3L. Acone-2 allozymes in the different developmental stages of the mosquito were characterized by thermostability, pH optima, effect of urea, EDTA, and p-chloromercuribenzoate. Natural populations collected from the Atlantic and Pacific coasts of Colombia were analyzed at 25 enzyme loci, and the analysis of the genetic structure of these populations indicated the presence of diagnostic allozyme differences. Verification of these diagnostic loci is underway and will be completed before conclusions will be drawn from the statistical analysis of the electrophoretic data.

Publications: 81/12 to 85/12
NO PUBLICATIONS REPORTED THIS PERIOD.

40.003* CRIS0135591
CHARACTERIZATION OF SYLVATIC AND DOMESTIC T. SPIRALIS ISOLATES BY DNA PROFILING AND ANTIGEN ANALYSIS

WORLEY D E; BURGESS D E; Veterinary Research Laboratory; Montana State University, Bozeman, **MONTANA** 59717.

Proj. No.: MONB00429Project Type: ANIMAL HEALTH
Agency ID: CSRS Period: 19 AUG 88 to 30 SEP 89

Objectives: To compare strains of Trichinella spiralis from various wild hosts and from domestic pigs using DNA sequence patterns and monoclonal antibodies.

Approach: Strains of Trichinella spiralis obtained from wild carnivores and domestic pigs will be maintained by periodic passage in Swiss Webster or deer mice. Muscle larvae reared in rodents will be collected by peptic digestion, washed in PBS, and used to prepare worm homogenates for electrophoresis and other analytical procedures, including restriction fragment length polymorphism (RFLP) analysis and polyacrylamide gel electrophoresis (SDS-PAGE). Portions of gels will be stained by Coomassie blue and silver nitrate and other portions will be used to prepare western blots. Deer mouse sera will be used to probe these blots.

Progress: 88/01 to 88/12. Six different Trichinella isolates from various sylvatic carnivores, and one isolate from a domestic pig were examined and compared by RFLP (restriction enzyme fragment length polymorphism) patterns of genomic DNA. Restriction enzyme fragments generated by the EcoRI restriction (Fig. 1) show a unique band of repetitive fragments in the Thai Pig isolate at about 1.8 kb which are not present in the sylvatic isolates. Conversely, there are unique bands in the sylvatic isolates at about 5.0 kb which are not present in the Thai Pig isolate. Patterns between 6 sylvatic isolates are nearly identical. Genomic DNA fragments generated by digestion with the Hind III restriction enzyme show unique bands in Thai Pig DNA at 1.5 kb, 1.8 kb, and 6.0 kb which are not present in the sylvatic isolates. A unique band occurs in lynx DNA at 9.8 kb which is not present in any of the other sylvatic isolates. Marked differences were also revealed in the antigens recognized between antigenic sources as well as by sera from mice infected with different strains when T. spiralis muscle larvae were separated by SDS-PAGE, electroblotted and probed with these sera.

Publications: 88/01 to 88/12
WORLEY, D.E., SEESEE, F.M. and ZARLENGA, D.S. 1988. Differential freezing resistance of T. spiralis isolates from MT & Alberta gray wolves w/observations on subspecific relationships of the MT biotype. Proc. Helm. Soc. Wash. sub.

40.004 CRIS0083930
IMMUNOLOGY - IMMUNOGENETICS

CYPESS R; DAVID C S; Preventive Medicine; Cornell University, Ithaca, **NEW YORK** 14853.
Proj. No.: NYCV-440-446 Project Type: STATE
Agency ID: CSVM Period: 01 JUL 80 to 31 DEC 87

Objectives: Map and characterize the inheritance of the H-2-linked genes which influence susceptibility and resistance to infection with Trichinella spiralis in the mouse, determine the developmental stages in the parasite's life history which are influenced by the genes identified and begin characterization of the specific cell types mediating immunity to this parasite.

Approach: Objectives will be addressed by studying infestions in congenic strains of mice which differ only at genes within the H-2 complex but demonstrate different levels of resistance to infection with T. spiralis. Alloantisera specific for the products of H-2-linked genes and for the Ly antigens expressed on the surface of functionally defined subpopulations of lymphoid cells, will be used to selectively deplete certain cell types from populations of cells isolated for study. These antiserum-treated cell populations, lacking specific subsets of immunoreactive cells, will then be tested to see if their in vitro proliferative response to Trichinella antigen has been altered or if they differ in their ability to adoptively transfer resistance to previously uninfected, syngeneic hosts. Altered activity following antiserum treatment will implicate cells bearing the appropriate specificity as important in contributing to the response being investigated.

Progress: 87/01 to 87/12. No further progress to report. This investigator has left Cornell University.

Publications: 87/01 to 87/12
NO PUBLICATIONS REPORTED THIS PERIOD.

40.005* CRIS0133059
FLOW CYTOMETRIC DETERMINATION OF INSECT DNA FOR IDENTIFICATION AND CONTROL OF INSECTS

JOHNSON J S; Entomology; Texas A&M University, College Station, **TEXAS** 77843.
Proj. No.: TEX06916 Project Type: HATCH
Agency ID: CSRS Period: 18 SEP 87 to 31 AUG 92

Objectives: 1) Develop flow cytometry and in particular total nuclear DNA content determination as a cytotaxonomic tool, for the recognition of host races, biotypes, and crytic species of insects. 2) Catalogue the nuclear DNA content of insects of economic interest. 3) Develop multiparameter DNA flow cytometry using dyes which are specific for total DNA, adenine and thymine rich repetitive DNA, and guanine and cytosine rich repetitive DNA. 4) Demonstrate the developmental pattern and mode of inheritance of DNA content variation for both euchromatic (unique) and heterochromatic (repetitive) insect DNA sequences. 5) Develop methodology to sort insect nuclei and chromosomes as an aid to gene cloning in insects.

Approach: Assay the total DNA, and sequence specific DNA content within and between individuals, populations, races, and species of economically important insects as an aid to monitoring insects of economic interest which are subjects of IPM control efforts.

CM 40

Progress: 88/01 to 88/12. We have now had one year of work with this unique new methodology for the study of heritable variation in insects. We have measured the DNA content of over 1500 individual fire ants including 3 native and two imported species. In addition, we have measured the DNA content of over 200 honey bees, nearly 300 boll weevils, plus smaller number of individuals from 10 other species. The results have been unexpected and exciting. In particular we find: DNA content is a diagnostic tool that aids in identification of species, populations and types. To date, very significant and predictable DNA content differences have been found between 5 fire ants species, between 4 species of boll weevil, and between the Africanized form of the honey bee, the European honey bee and the F-1 hybrid between the two forms. DNA content variation exists within and among some populations. The most extreme variation we have observed occurs in a fire ant population in Walton Co., Georgia, were 129 of 515 males, females, and workers show 3N or triploid DNA amounts. The remainder are diploid or haploid as expected. The smallest significant variation occurs between populations of the boll weevil Anthonomus grandis where DNA content changes are associated with different host plants. DNA change occurs during development. Male fire ants are haploid during early development, but double their DNA before maturity. Boll weevils are largely 2N throughout their life, although in 1/4 to 3/4 of their cells may have 15 to 20 percent additional DNA.

Publications: 88/01 to 88/12

JOHNSTON, J.S., ELLISON, J.R. and VINSON, S.B. 1989. Flow cytometric determination of insect DNA as an aid to the description, identification, and control of the imported fire ant. Vinson SB McCOWN JL (eds.) Proc. Imported Fire Ant Sympo.

JOHNSTON, J.S. and ELLISON, J.R. 1989. DNA heterogeniety within and among fire-ants of the genus Solenopsis in southern United States: Evidence from flow cytometry. Submitted to Cytometry.

JOHNSTON, J.S. and ELLISON, J.R. 1988. Triploidy in a polygynous population of the imported fire ant Solenopsis invicta in Walton Co., Georgia. Submitted to Hereditas.

ELLISON, J.R., VINSON, S.B. and JOHNSTON, J.S. 1989. A method for determination of DNA content of nuclei of insects by flow cytometry. Submitted to Cytometry.

ELLISON, J.R., VINSON, S.B. and JOHNSTON, J.S. 1989. Acquired somatic diploidy in males of the imported fire ant. Submitted to Canadian Jn. Genet. and Cytogenet.

CM 63 BIOLOGICAL CELL SYSTEMS

63.001* CRIS0134012
GENE SYNTHESIS AND EXPRESSION OF PROTEIN PRODUCTS FOR NATURAL AND MUTANT HEAT-STABLE ENTEROTOXIN

TRUMBLE W R; Bacteriology & Biochemistry; University of Idaho, Moscow, **IDAHO** 83843.
Proj. No.: IDA00911 Project Type: HATCH
Agency ID: CSRS Period: 01 JAN 88 to 31 DEC 92

Objectives: Constructing a synthetic gene and expressing active heat-stable enterotoxin (ST-1) and inactive mutant analog peptides. The synthetic gene(s) will be used to attempt to make the enterotoxin immunoreactive for vaccine development and to allow homologous recombination experiments to produce an infective E. coli incapable of producing active enterotoxin for use as a veterinary treatment to prevent enterotoxigenic EV coli diarrheal disease.

Approach: The published amino acid sequence of the ST-1 peptide has been back translated into nucleotide sequence. Several important design features have been incorporated into the gene design. Synthetic oligonucleotides will be constructed containing mutations for specific amino acids and after combining the oligonucleotides to produce double strand ST-1 genes, DNA probes will be used to isolate genes encoding native and mutant ST-1 peptides.

Progress: 88/01 to 88/12. The E. coli toxin, STIa, causing infant and traveler's diarrhea in humans and collibacilosis in cattle, sheep and pigs, is an 18 amino acid peptide which is too small to be recognized by the immune system. At present, there is no effective vaccine or prophylactic treatment against STIa-mediated diarrheal disease. We are using two approaches to provide protection against the effects of this toxin. First, a synthetic gene has been constructed which links repeating units (encoding 17 of the 18 amino acids) of the STIa gene to produce a "multimeric" gene capable of expressing a large, antigenic protein which should have multiple epitopes in common with the native toxin. We have cloned "multimeric genes" which encode from two to ten linked-copies of the monomeric "core" sequence. The recombinant proteins will be tested for immune response, toxicity and neutralizing ability. Second, we have constructed a synthetic analog of the toxin gene. Putatively important amino acids have been deliberately modified through changes at the DNA level to produce a non-toxic STIa-analog protein. The "non-functional" gene-encoded protein will be compared to activity of a synthetic "wildtype" gene we have constructed. Using insertional sequences, we will attempt to insert this non-functional gene in place of the active toxin-producing gene to construct a biological control organism which would be capable of colonizing the gut but would be incapable of producing an active STIa-toxin.

Publications: 88/01 to 88/12
No publications reported this period.

63.002 CRIS0074454
DEFENSIVE RESPONSES IN INSECTS AND PLANTS

DUNN P E; Entomology; Purdue University, West Lafayette, **INDIANA** 47907.
Proj. No.: IND058047 Project Type: HATCH
Agency ID: CSRS Period: 15 NOV 88 to 30 SEP 93

Objectives: Analysis of the structure, synthesis, and function of hemofibrin. Characterization of peptidoglycan regulated protein synthesis and feeding inhibition. Determination of the structure of antibacterial protein genes and characterization of mechanisms regulating their expression. Characterize antibacterial protein synthesis by Malpighian tubules. Analysis of the mechanism of parasitoid-induced hemocyte dysfunction. Transformation of a plant with CLP gene to confer increased resistance to bacterial pathogens.

Approach: Protein structural analysis, molecular cloning, nucleotide sequence analysis, quantitative binding assays, analysis of second messenger titer and metabolism, monitoring bio-activity, in vitro transcription run-off assays, in vitro organ culture, in situ hybridization, immunocytochemical analysis, plant transformation, Northern hybridization analysis, assay of susceptibility of transformed plants to bacterial pathogens.

Progress: 87/10 to 88/09. We have identified a 22kD protein, hemofibrin, which is a constitutive component of the hemolymph of immunologically naive larvae of Manduca sexta. When hemolymph contacts wounded tissue surfaces, hemofibrin rapidly aggregates to form a fibrous coagulum like that formed from the vertebrate protein fibrin during blood clotting. The fibrous coagulum formed from hemolymph hemofibrin serves as a net and traps hemocytes present in the hemolymph which flows from the wound. The resulting hemocyte/hemofibrin coagulum appears to form the primary clot which plugs wound openings. Hemofibrin has been purified in native (unassembled) form hemolymph of M. sexta. Analysis of hemofibrin primary structure and of the aggregation process are in progress. Previous results from RNase protection experiments demonstrated that treatment of M. sexta larvae with peptidoglycan induced lysozyme, cecropin-like peptide, and attacin-like protein (ALP) genes in Malpighian tubules, resulting an accumulation of RNA transcripts from these genes. Using in vitro culture techniques, we have confirmed the peptidoglycan-regulated synthesis and secretion of lysozyme and ALP proteins by Malpighian tubule tissue. Studies of the suppressed antibacterial response in M. sexta parasitized by Cotesia congregata have demonstrated that a functional hemocyte-mediated response is not required for the development or maintenance of an "immunized" state after injection of bacteria or peptidoglycan.

Publications: 87/10 to 88/09
DAI, W. (1988). Structure and regulation of the synthesis and secretion of an attacin-like protein from larvae of Manduca sexta. Ph.D. Thesis, Purdue University, W.

Lafayette, IN.
ROSS, D. R. (1988). Effects of parasitism by Cotesia congregata on the antibacterial responses of the tobacco hornworm, Manduca sexta. Ph.D. Thesis, Purdue University, W. Lafayette, IN.
KANOST, M. R., W. DAI, and P. E. DUNN. (1988). Peptidoglycan fragments elicit antibacterial protein synthesis in larvae of Manduca sexta. Arch Insect Biochem. Physiol. 8:147-164.
GENG, C. and P. E. DUNN. (1988). Hemostasis in larvae of Manduca sexta: formation of a fibrous coagulum by hemolymph proteins. Biochem. Biophys. Res. Comm. 155:1060-1065.
DICKINSON, L. V. RUSSELL and P. E. DUNN. (1988). A Family of bacteria-regulated, cecropin D-like peptides from Manduca sexta. J. Biol. Chem., in press.

63.003* CRIS0088114
PHYSICAL, CHEMICAL, EVOLUTIONARY AND STATISTICAL STUDIES OF THE BOVINE GENOME

BLAKE R D; Biochemistry; University of Maine, Orono, **MAINE** 04469.
Proj. No.: ME08405 Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 82 to 30 SEP 87

Objectives: The proposed work is designed to increase our knowledge of aspects of structure and dynamic behavior of the bovine genome. We will endeavor to determine the precise number of repetitive sequence groups, their length, base composition and sequence, the sequence divergence throughout each repetive sequence group, their evolutionary origins and ages, and the fractional amounts of each group in total bovine genomic DNA.

Approach: Total bovine genomic DNA will be fractionated by preparative Hg Cs(2)SO(4) density gradient contrifugation. The G-C rich satellite ands will be subjected to restriction endonuclease mapping, and the restriction fragments isolated by preparative electrophoresis and RPC-5 HPLC. These fragments will be amplified by cloning with pBR322 as a vehicle, and subcloned to separate fragments of unique sequence in the divergent population. Both cloned and subcloned fragments will be analyzed for the extent of sequence divergence and age by the method of high resolution thermal dispersion analysis. Subcloned fragments will be sequenced by the Maxim & Gilbert method.

Progress: 86/10 to 87/09. Bovine satellite I DNA has been purified to >90%, and satellite II to >60%. Clones of pBR322 recombinants of these satellites have been obtained. A high resolution derivative melting curve of the 1402 bp satellite I DNA corresponds perfectly in both temperature and breadth with a large subtransition in the melting profile for total genomic CT-DNA. From the relative integrated areas under the satellite and total CT-DNA melting curves, we estimate this satellite to contribute 15% to the total DNA mass of the bovine genome, which is 2 times more than previous estimates. This translates to a frequency of almost 500,000 coies of the 1402 bp repeat in the genome. A theoretical melting curve for satellite I DNA, produced from the published sequence of a single clone is narrower, but occurs at almost the same T(m). The half band width for the theoretical curve computed from a specific sequence is only 0.180 C, whereas the half band width for the population is 0.580 . The curve for the population of satellite I sequences is symmetrical and broader by 0.367 due to the sequence divergence that has accrued over evolutionary time in an apparent random manner throughout the population. The base composition and standard deviation of the DNA producing the large subtransition is 0.584 +/- 0.0089. We have estimated that this subtransition is produced from the dissociation of a segment of DNA amounting to differece 72% of satellite I.

Publications: 86/10 to 87/09
BLAKE, R.D. 1987. "Cooperative Lengths of DNA During Melting", Biopolymers 26, 1063-1074.
BLAKE, R.D. and EARLEY, S. 1986. "Distribution and Evolution of Sequence Characteristics in the E.coli Genome", J. Biomol. Structure and Dynamics 4, 291-307.
QURESHI, S. and BLAKE, R.D. 1987. "Repetitive Sequences in the Bovine Genome", Me.Biomed.Sci.Symp.
BLAKE, R.D. and DELCOURT, S.G. 1987. "Loop Energy in DNA" Biopolymers 27,001-015.
BLAKE, R.D. and HELEK-DAY, S. 1986. "Parameters in the Theoretical Model for Nonhelical DNA Structures", Macromolecules-86 (Ed. R. Epton), Oxford, p. 143.
BLAKE, R.D. and DELCOURT, S.G. 1987. "Elasticity of DNA in Nonhelical Loops", Fed. Proc. 46,1960.
BLAKE, R.D., HINDS, P.W., EARLEY, S., HILLYARD, A.L. and DAY, G.R. 1986. "Evolution and Functional Significance of the Bias in Codon Usage", in Biomolecular Stereodynamics IV (Eds. R.H. Sarama and M.H. Sarma), Adenine Press, pp. 271-286.

63.004 CRIS0049420
MOLECULAR AND BIOCHEMICAL GENETICS OF TRICHOGRAMMA AND HELIOTHIS FOR BIOSYSTEMATICS AND BIOCONTROL

HUETTEL M D; HUNG A C F; Beneficial Insect Intro Lab Insect Iden & Bene Insect Inst; Beltsville Agr Res Center, Beltsville, **MARYLAND** 20705.
Proj. No.: 1275-21240-001-00D
Project Type: INHOUSE
Agency ID: ARS Period: 01 OCT 85 to 30 SEP 90

Objectives: Develop and adapt molecular, biochemical, and other genetic technologies for the systematics and identification of Trichogramma and Heliothis spe- cies and to the biological control of Heliothis.

Approach: Characterize populations and species of Trichogramma parasitoids and Helio-this moths using isozyme analyses, genetic maps of mitochondrial DNA (mtDNA), and advanced chromosomal techniques as appropriate. Define specieslimits within Trichogramma by cross-mating experiments. Investigate

methodsfor the induction of mitochondrially-based inherited male sterility in H. zea. Augment control of H. virescens by conferring virus resistance upon released male-sterile backcross hybrids. Develop monoclonal antibodies against insect extracts for use in ELISA and RIA. Beltsville, MD, Bg 225; BL-1; approval request in preparation. Scientists and technicians associated with project: M. Huettel, E. Quinn, W. Sheppard.

Progress: 88/01 to 88/12. The development an of ELISA kit for field identification of Africanized honey bees is experiencing expected difficulties, because subspecies-specific antigens are not yet available. Different methods of immunosuppression are being tried to overcome these difficulties. The first case of parasitic hymenoptera culture changing from arrhenotoky to thelytokoy was documented with evidence from analysis of 23 isozyme loci. The MS was submitted for peer review. Developmental stage-specific isozyme loci were identified in the marsh fly, Sepedon fuscipennis. The information is essential for the biosystematic study in the S. fuscipennis species complex. A total of 24 lots of Trichogrammatidae from 12 states and 3 countries were received and identified. Progress has been made in adapting techniques for extraction of mtDNA from single, frozen heliothine adults for species' comparisons using Southern blots of mtRFLPs visualized by nonradioactive probes. Small sample sizes of rare species continue to be a problem, however. The genetic relationships between previously described genes controlling pheromone production in females and response in males of the European corn borer were described in field and lab studies. A rapid molecular technique for identification of Africanized honey bees using restriction enzyme analysis of mitochondrial DNA was developed.

Publications: 88/01 to 88/12

HUNG, A.C.F., DAY, W.H. and HEDLUND, R.C. 1988. Genetic variability in arrhe notokous and thelytokous forms of Mesochorus nigripes (Hym.:Ichneumonidae) . Entomophaga 33: 7-15.

HUNG, A.C.F. 1988. Taxonomic treatment of thelytokous forms in parasitic Hymenop tera. In: V.K. Gupta, ed., Advances in Parasitic Hymenoptera Research, pp. 163-167.

KLUN, J.A. and HUETTEL, M.D. 1988. Genetic regulation of sexpheromone production and response: Interaction of sympatric types of European corn borer, Ostrinia n ubilalis (Lepidoptera:Pyralidae). Jour. Chem. Ecol. 14:2047-2061.

LASTER, M., CARPENTER, J., HUETTEL, M., et al. 1988. Auto- cidal systems for He liothis control. In: Herzog, S. et al., eds., Theory & tactics of Heliothis PopM anag.:III. Emerging control tactics & tech. S. Coop. Ser Bull. 337:74-103.

SHEPPARD, W.S. and HUETTEL, M.D. 1988. Biochemical genetic markers, intraspecif ic variation, & population genetics of the honey bee, Apis mellifera. In: Needh am et al. eds., Africanized Honey Bees & Bee Mites, E. Horwood Ltd, Eng.

63.005 CRIS0048495

ARTHROPOD VIRUSES: CHARACTERIZATION, GENETICS AND REPLICATION IN VIVO AND IN VITRO

VAUGHN J L; TOMPKINS G J; ADAMS J R; Insect Pathology Lab Plant Protection Inst; Beltsville Agr Res Center, Beltsville, **MARYLAND** 20705.
Proj. No.: 1275-22240-002-00D
Project Type: INHOUSE
Agency ID: ARS Period: 29 JUN 83 to 29 JUN 88

Objectives: To characterize viruses from insect pests and evaluate their potential for use in pest management. To determine biochemical processes of virus invasion, replication and virulence and the genetic factors controlling these processes. To develop the necessary in vitro systems for the isolation, replication and production of insect pathogenic viruses.

Approach: Characterization will be done by a combination of light and electron microscope studies and by biochemical analysis of the structural components of the virus. Genetic relatedness to other viral isolates from the same or similar insect species will be determined by restriction endonuclease analysis of the viral genome and by nucleic hybridization tests. Genetic manipulation will be either by classical methods of viral recombination or the isolation, cloning and transfer of specific genes using the methods of plasmid insertion, splicing and ligation for genetic engineering. In vitro studies will involve development of new cell lines, and studies of the effects of cell nutrition and metabolism of virus replication.

Progress: 87/01 to 87/12. In cooperation with Lim Technology, Richmond, Va., an encapsulation process was tested that did not inactivate baculoviruses. Field studies indicate significant increase in activity of viruses encapsulated with selected dyes compared with viruses encapsulated without dyes or unencapsulated. These results are of interest to scientists and to those companies formulating these microorganisms. Restriction endonuclease mapping of the gypsy moth virus genome was completed and the site of the polyhedrin gene identified. A cell line that permitted partial replication was identified, permitting studies on biochemical factors limiting host range of the nuclear polyhedrosis viruses. A new cell line derived from fat body tissue of the gypsy moth produces significantly more polyhedra when infected with a highly virulent strain of gypsy moth virus. The cell line was adapted to a readily available tissue culture medium (TC-100) to increase its usefulness for production of the virus. Studies with some commercially available serum replacements demonstrated that one of these, CPSR-3, Sigma Chemical, was satisfactory. Cell growth and baculovirus production were equivalent to that in serum supplemented media at 1/3 the cost. Studies demonstrated that there is more variation in virulence of viruses produced in cell cultures than for the same viruses produced in insects. These results are of interest primarily to other scientists.

Publications: 87/01 to 87/12

LYNN, D.E., FELDLAUFER, M.F. and LUSBY, W.R. 1987. Isolation and identification of 20-hydroxyecdysone from a lepidopteran continuous cell line. Arch. Insect Biochem. Physiol. 5:71-79.

MCCLINTOCK, J.T., LYNN, D.E., DOUGHERTY, E.M. and SHIELDS, K. 1987. Embryonic and fat body cell cultures from gypsy moth: characterization and virus susceptibility. In Vitro Cell Dev. Biol. 23:62A. (Abstract).

LYNN, D.E. and OBERLANDER, H. 1986. Obtainment of hormonally sensitive cell lines from imaginal discs of Lepidoptera species. Tech. in the Life Sci., C1, In Vitro Invertebr. Hormones and Genes, C213, 1-12 Els. Sci. Pub., Ireland Ltd.

TOMPKINS, G.J., DOUGHERTY, E.M., ADAMS, J.R., and DIGGS, D. 1987. Changes in virulence of NPV when propagated in alternate noctuid (Lepidoptera:Noctuidae) cell lines and hosts. Accepted by J. Econ. Entomol., Dec. 11, 1987.

DOUGHERTY, E.M., MCCLINTOCK, J.T., and SHIELDS, K. 1987. In vivo infection of L. dispar with a nuclear polyhedrosis virus of Autographa californica (AcNPV). VII Int. Congress of Virology, Aug. 14-19, 1987, Edmonton, Canada p 208 (Abstr).

VAUGHN, J.L., ADAMS, J.R., DOUGHERTY, E.M. and MCCLINTOCK, J.T. 1987. The use of serum replacements in medium for the in vitro production of an insect NPV. VII Int. Congress of Virology, Aug. 14-19, 1987, Edmonton, Canada p 207 (Abstr).

DOUGHERTY, E.M. 1987. Insect viral control agents. Developments in Industrial Microbiology 28:63-75.

63.006 0058005

CHROMATOGRAPHY OF BIOLOGICALLY IMPORTANT MOLECULES AND APPLICATIONS

GEHRKE C W; Biochemistry; University of Missouri, **MISSOURI** 65211.

Proj. No.: MO-00009 Project Type: HATCH
Agency ID: CSRS Period: 01 JUL 85 to 30 SEP 89

Objectives: Our research will center on DNA methylation and the m C content of human DNA sequences transcribed to different extents, and whether m C is actually found in dinucleotides other than m CpG and to examine other thermophilic organisms to determine their N -methylcytosine content. Research will be continued on advancement of high resolution HPLC for 60 modified nucleosides in tRNA and the structural characterization by UV and mass spectrometry of representative and unknown modified nucleosides. Studies will be made on chromatography and characterization of cap structures in mRNA and investigations on development of high resolution separation of RNA and DNA oligomers after base specific enzymatic hydrolysis.

Approach: Our approach is the advancement and further development of high resolution HPLC methods, multiwavelength UV spectral analysis, and interfaced capillary GC-MS for the accurate measurement and characterization of a wide array of major and modified nucleosides in DNA, tRNA, mRNA, oligomers; and for the development of pattern recognition studies by HPLC of enzymatic hydrolysates of peptides and proteins.

Progress: 88/01 to 88/12. We report standard RPLC-UV methologies for the analysis of more than 65 nucleosides in a single run with "run to run" peak retention variations of less than 1%. A complete nucleoside composition can be achieved with less than 0.5 microgram of RNA. Complete chromatographic protocols, nucleoside columns and parameters of operation are given for high resolution, high speed, and high sensitivity chromatography. Three unfractionated tRNAs are given as sources of reference compounds, also an extended enzymatic hydrolysis method. We are the first to report the presence of phosphorylated P-Ribosyl Adenosine in T-(psi)-Stem of yeast methionine initiator tRNA with characterization by HPLC-UV, MS, and NMR. Examples are given of unique applications of our advanced nucleoside methodology to biochemical and biomedical investigations including codon-anticodon discrimination and tRNA structural content a new method for quantitation of nucleosides in serum, and the reduced genomic content of m C in colonic neoplasias. These new research tools will have important contributions to biochemical and medical research.

Publications: 88/01 to 88/12

MCENTIRE, J.E., KUO, K.C., SMITH, M.E., STALLING, D.L., RICHENS, J.W. JR., ZUMWALT, R.W., FEHRKE, C.W., and PARPERMASTER, B.W.: Classification of Lung Cancer Patients and Controls by Chromatography of Modified Nucleosides in Serum.

KUO, K.C., PHAN, D.T., WILLIAMS, N., and GEHRKE, C.W.: Ribonucleosides in Serum and Urine by High Resolution Quantitative RPLC-UV Method. J. Chromatogr., in press.

PAPERMASTER, B.W., MCENTIRE, J.E., SMITH, M.E., BROWNSON, R.C., RICHENS, J.W. JR., KUO, K.C., and GEHRKE, C.W.: Increased Lung Cancer Mortality in a Rural Lead and Zinc Mining area: A Multivariate, Case-Control Study of Personal History Vi.

FEINBERG, A.P., GEHRKE, C.W., KUO, K.C. and EHRLICH, M.: Reduced Genomic 5-Methylcytosine Content in Human Colonic Neoplasis. Cancer Research 48:1159:1161, (1988).

63.007 CRIS0085099

REGULATION OF GENE EXPRESSION DURING EUCARYOTIC DEVELOPMENT

BEWLEY G C; Genetics; North Carolina State University, Raleigh, **NORTH CAROLINA** 27695.

Proj. No.: NC03762 Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 81 to 30 SEP 87

Objectives: Determine any structural differences betewwn the isozymes of glycerol-3-phosphate dehydrogenase in Drosophila. Determine the manner in which the expression of GPDH isozymes are controlled during development. Isolate and chracterize genetic variants affecting the developmental

program of GPDH expression.

Approach: Each purified isozyme is to be subjected to a structural analysis including tryptic digestion, generation of peptide maps, purification of peptides from each map with a subsequent compositional and sequence analysis. Genetic variants of the developmental program of GPDH will be characterized by mapping studies, protein turnover studies and an analysis of the steady-state protein.

Progress: 78/10 to 87/09. Glycerol-phosphate dehydrogenase in Drosophila melanogaster consists of a family of three isozymes which exhibit a unique temporal and tissue-specific pattern of expression. Even though these isozymes are encoded by the same structural gene, they differ in the amino acid sequence at the C-terminal end. We have isolated both genomic and cDNA clones in order to examine the structure of the 3'-end of this gene and its transcriptional products. The results of this analysis demonstrate that this gene represents a complex transcriptional unit whereby the isozymic forms of GPDH are encoded by three classes of transcripts each generated by developmentally regulated 3'-end formations and alternate splicing pathways of the pre-mRNA. We have just completed a genetic screen which has resulted in the isolation of six null mutants for the enzyme catalase. These mutants are currently being characterized. The mutants will provide us with the raw material to analyze the role of catalase in oxygen free radical metabolism and the role of oxygen radicals in promoting genetic damage.

Publications: 78/10 to 87/09

SHAFFER, J. B., SUTTON, R. B. and G. C. BEWLEY. 1987. Isolation of a cDNA clone for murine catalase and analysis of an acatalasemic mutant. J. Biol. Chem. 262:12908-12911.

CM 64 EXPERIMENTAL DESIGN AND STATISTICAL METHODS

64.001 CRIS0097030
VACCINIA VIRUS: EUKARYOTIC CLONING AND EXPRESSION VECTOR

HRUBY D E; Microbiology; Oregon State University, Corvallis, **OREGON** 97331.
Proj. No.: ORE00034 Project Type: STATE
Agency ID: SAES Period: 15 SEP 85 to 14 SEP 90

Objectives: To develop improved methods of introducing foreign DNA into vaccinia virus. To use this methodology to construct recombinant viruses which may be used as vaccines or to produce bioreactive compounds.

Approach: DNA-mediated marker transfer techniques will be employed to insert genes of interest into vaccinia virus. Recombinant viruses will be isolated and characterized as to their genome structure, transcription of the foreign DNA, and expression of new proteins. The ability of recombinant viruses to induce appropriate immune reactions in vivo will be evaluated. Where indicated, the DNA sequence of the virus will be modified to improve the level of gene expression by the recombinant viruses.

Progress: 88/01 to 88/12. Vaccinia virus is being developed as an efficient system for the production of a variety of biopharmaceutical products as well as a recombinant vaccine vector. The experiments carried out in our laboratory at OSU are directed towards improving methods of recombinant virus construction and analyzing the biological properties of the imitant viruses that we are able to assemble. Results for the current year are: (1) Several regions of the Vv genome have been sequenced and subjected to rigorous molecular genetic analyses. The results of these experiments should provide information regarding both additional insertion sites for foreign genes and the location of viral promoter elements which can be used to express the passenger genetic information. (2) Genes encoding plant virus protease genes have been expressed in VV in a functional form. These vectors are currently being used to explore an experimental strategy for controlling the spread of plant pathogens in the field. (3) a recombinant vaccine against Streptococcus pyogenes has been developed. Further analysis of this recombinant may provide information which will allow the development of other vaccines against other animal and human pathogens which exhibit immunological diversity.

Publications: 88/01 to 88/12

HODGES, W.M. and HRUBY, D.E. (1987). Cell-free translation of a chimeric eukaryotic-prokaryotic message yields functional chloramphenicol acetyltransferase. Analytical Biochemistry 160:65-67.

WEINRICH, S.L. and HRUBY, D.E. (1987). Non-coordinate regulation of a vaccinia virus late gene cluster. J. Virol. 61:639-645.

FRANKE, C.A. and HRUBY, D.E. (1987). Association of non-viral proteins with recombinant vaccinia virus. Archives of Virology 94:347-351.

ROSEMAN, N.A. and HRUBY, D.E. (1987). Nucleotide sequence and transcript organization of a region of the vaccina virus genome which encodes a constitutively expressed gene required for DNA replication. J. Virol. 61:1398-1406.

FRANKE, C.A. and HRUBY, D.E. (1987). Quantitative assay of recombinant vaccinia virus-encoded neomycin phosphotransferase in infected eukaryotic cell lysates. J. Virol. Methods 16:147-154.

WILSON, E.M. and HRUBY, D.E. (1987). Use of single-stranded insertion vectors (SSIV) to introduce foreign genes into the vaccinia virus genome. Nuc. Acids. Res. 15:4690.

MINER, J., WEINRICH, S.L. and HRUBY, D.E. (1988). Molecular dissection if cis-acting regulatory elements from 5'-proximal regions of VV late gene cluster. J. Virol. 62:297-304.

65.001 CRIS0092320
PHYSIOLOGY AND GENETICS OF LARVICIDAL TOXIN PRODUCTION BY BACILLUS SPHAERICUS

BAUMANN P; Agri Bacteriology; University of California, Davis, **CALIFORNIA** 95616.
Proj. No.: CA-D*-BAC-4403-H Project Type: HATCH
Agency ID: CSRS Period: 12 APR 88 to 30 SEP 92

Objectives: Over-production of the 42 and 51 kDa toxins of Bacillus sphaericus in Escherichia coli. Determination of the toxicity of the recombinant produced proteins to larvae of Culex pipiens and to tissue culture-grown cells of Culex quinquefasciatus. Study of the pathway of activation of these proteins in larvae of C. pipiens. Cloning of the gene coding for the 125 kDa toxin of B. sphaericus.

Approach: Change of the sequence adjacent to the beginning ATG codon coding for the 42 & 51 kDa protein by in vitro mutagenesis so as to create an Eco RI site. Insertion of these genes into the Eco RI site of pKK233-3, an expression vector. Removal by in vitro mutagenesis of 11 amino acids from the N-terminus and 21 amino acids from the C-terminus of the DNA coding for the 42 kDa protein. Insertion of the DNA coding for the resulting 39 kDa protein into the expression vector pKK233-3.

Progress: 88/01 to 88/12. The nucleoside sequences of a 3,479-base-pair HindIII DNA fragment from Bacillus sphaericus 2362 and a 2,940-base-pair fragment from strain 2297 were determined; only minor differences were detected between them. Each contained two open reading frames coding for proteins of 51.4 and 41.9 kilodaltons. Both proteins were required for toxicity to larvae of the mosquito Culex pipiens. A 3080-base-pair Kpn I-HindIII DNA fragment from Bacillus sphaericus 2362 coding for 51 and 42 kilodalton mosquitocidal proteins was cloned into B. subtilis DB104 using the vector pUB18. In B. subtilis these proteins were not detected during vegetative growth but were expressed during sporulation at levels comparable to that found in B. sphaericus.

Publications: 88/01 to 88/12

BAUMANN, L., A. H. BROADWELL, and P. BAUMANN. (1988). Sequence analysis of the mosquitocidal toxin genes encoding 51.4- and 41.9-kilodalton proteins from Bacillus sphaericus 2362 and 2297. Journal of Bacteriology 170, 2045-2297.

65.002 0096410
EVOLUTIONARY RELATIONSHIP OF THE PROCARYOTIC ENDOSYMBIONTS OF APHIDS

BAUMANN P; Agri Microbiology; University of California, Davis, **CALIFORNIA** 95616.
Proj. No.: CA-D*-MIC-4555-H Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 89 to 30 SEP 94

Objectives: We are interested in the elucidation of evolutionary relationships of procaryotic aphid endosymbionts. Relationships will be established by cloning the genes coding for the 16S rRNA of the endosymbionts. The oligonucleotide sequence will be determined and used to construct evolutionary trees based on sequence similarity. The results will allow us to distinguish between a single ancient infection of an aphid ancestor and multiple infection of different lineages of different procaryotes.

Approach: Using selected oligonucleotide primers and the polymerase chain reaction the 16S rDNA from the endosymbionts of aphids will be amplified and cloned into M13mp18/19. Subsequently by using primers to conserve regions of the 16S rDNA we will determine the sequence of the cloned genes.

Progress: 88/01 to 88/12. The pea aphid (Acyrthosiphon pisum Harris) harbors two morphologically distinct procaryotic intracellular symbionts. The genes for the 16S ribosomal RNA (rRNA) from these symbionts have been cloned and sequenced. Comparisons with sequences of 16S rRNAs from selected procaryotes indicate that the two symbionts are evolutionarily distinct from each other and are members of the (gamma)-3 subdivision of the Proteobacteria. One of the symbionts is a member of the Enterobacteriaceae while the other constitutes a lineage distinct from these organisms. Both symbionts appear to have one copy of their rRNA operon.

Publications: 88/01 to 88/12
No publications reported this period.

65.003 CRIS0099148
HORMONE REGULATED GENE ACTIVITY AT METAMORPHOSIS IN DROSOPHILA MELANOGASTER

NATZLE J E; Agri Zoology; University of California, Davis, **CALIFORNIA** 95616.
Proj. No.: CA-D*-AZO-4665-H Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 86 to 30 SEP 91

Objectives: Identification and cytological localization of the gene product of a hormone-inducible gene expressed in Drosophila during metamorphosis. Construction of a synthetic antigen representing the gene product, production of an antiserum, and determination of the antigen distribution in Drosophila tissues.

Approach: Combine immunological techniques with recombinant DNA technology. The protein antigen will be produced by fusing a portion of a cloned Drosophila gene to a gene encoding a bacterial protein in an E. coli expression vector. A polyclonal antiserum that recognizes the fusion protein will be used to detect the Drosophila antigen by immunoprecipitation and immunocytochemistry.

Progress: 88/01 to 88/12. The overall objective of this research is the identification and cytological localization of the product of a Drosophila melanogaster gene (IMP-E1) involved in imaginal disc development at metamorphosis. Holometabolous insects pass through a critical developmental period at metamorphosis when adult epidermal structures and appendages are formed from relatively undifferentiated precursor cells (imaginal

discs) in response to insect steroid hormones. Our work should lead to a better understanding of the regulation and function of genes required during insect metamorphosis. During this research period we have succeeded in placing a portion of a cDNA clone representing coding region of the IMP-E1 gene into a series of bacterial expression vectors (pWR-590, 590-1, 590-2). Analysis of proteins produced from bacterial hosts harboring the recombinant vectors suggests that one of the set of constructions is producing a fusion protein. We are currently determining the DNA sequence of the cDNA to verify that the orientation and reading frame of the fusion construction are correct. We have also started to collect DNA sequence information (and predicted amino acid sequence) from other regions of the cDNA to facilitate analysis of the properties and function of the IMP-E1 gene product.

Publications: 88/01 to 88/12
No publication reported this period.

65.004 CRIS0066506
EVOLUTIONARY GENETICS OF NATURAL POPULATIONS

AYALA F J; Genetics; University of California, Davis, **CALIFORNIA** 95616.
Proj. No.: CA-D*-GEN-3169-H Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 86 to 30 SEP 87

Objectives: To ascertain the amount of genetic variation in natural populations. To evaluate the processes responsible for maintaining genetic variation in natural populations. To ascertain the prevailing modes and ways by which new species arise.

Approach: Laboratory studies with Drosophila flies, using electrophoresis, protein purification, "fingerprinting", amino acid sequencing, and related techniques as well as the standard procedures of genetic analysis.

Progress: 86/01 to 87/09. We have analyzed the enormous fluctuations in the rate of evolution of the gene coding for superoxide dismutase, fluctuations that are inconsistent with the hypothesis of a molecular evolutionary clock. We have developed a model of density-dependent population growth and shown experimentally that Drosophila populations "adjust" their rate of development so as to maximize the probability of survival under conditions of strong competition for limited resources. We have shown that the rates of evolution of mitochondrial DNA differ by a factor of two or three between closely related evolutionary lineages of Drosophila.

Publications: 86/01 to 87/09
MOYA, A., GONZALEZ, F. and AYALA, F.J. 1986. Intra- and intergenotypic competition in Drosophila melanogaster: effects of density on larval survival and rate of development. Genetica 70:59-67.
MOYA, A., GONZALEZ, F., BOTELLA, L.M. and AYALA, F.J. 1986. Density-dependent pre-adult mortality in Drosophila: A theoretical model and experimental tests. Acta Oecologica, Generalis 7:231-242.
AYALA, F.J. 1986. On the virtues and pitfalls of the molecular evolutionary clock. J. Heredity 77:226-235.
LATORRE, A., MOYA, A. and AYALA, F.J. 1986. Evolution of mitochondrial DNA in Drosophila subobscura. Proc. Nat. Acad. Sci. U.S.A. 83:8649-8653.
MARINKOVIC, D. and AYALA, F.J. 1986. Genetic variation for rate of development in natural populations of Drosophila melanogaster. Genetica 71:123-132.
MARINOVIC, D. and AYALA, F.J. 1986. Selection for different rates of embryonic development in Drosophila melanogaster and Drosophila simulans. Genetica 18:205-219.
CLUSTER, P.D., MARINKOVIC, D., ALLARD, R.W. and AYALA, F.J. 1987. Correlations between development rates, enzyme activities, ribosomal DNA spacer-length phenotypes, and adaptatioon in Drosphila melanogaster. Proc. Nat. Acad. Sci. U.S.A.

65.005 CRIS0002658
HORMONAL REGULATION OF GENE FUNCTION IN INSECT DEVELOPMENT

FRISTROM J W; Genetics; University of California, Berkeley, **CALIFORNIA** 94720.
Proj. No.: CA-B*-GEN-2347-H Project Type: HATCH
Agency ID: CSRS Period: 03 OCT 85 to 30 SEP 90

Objectives: A better understanding of the mechanisms by which the insect steroid molting hormone, ecdysone, causes morphogenesis (formation of appendages) and differentiation (cuticle formation) of embryonic tissues (imaginal discs) of Drosophila melanogaster.

Approach: The receptor protein for ecdysone will be purified and characterized. Recombinant DNA techniques will be used to clone genes whose products putatively mediate morphogenesis and/or differentiation. Antibodies will be raised against the encoded proteins to allow immunochemical EM localization in cells. Mutants will be produced to confirm the function of the cloned gene.

Progress: 88/01 to 88/12. Studies continue on the effects of ecdysones (steroid molting hormones) on Drosophila imaginal disc biosynthesis and development including morphogenesis to form adult appendages and differentiation to form a cuticle. (1) An ecdyson-responsive regulatory gene (Br-C) has been found by sequence analysis to encode a protein with "zinc fingers" and is presumed to be a DNA-binding protein that regulates gene expression necessary for morphogenesis. (2) An ecdysone-responsive gene (Imp-E2) has been found to encode an apically secreted protein that may function either in morphogenesis or in the deposition of the cuticle or both. (3) A mutant that interferes with morphogenesis apparently disrupts a gene ((beta)-3 tubulin) for a specific (beta)-tubulin. (4) Additional mutants that disrupt disc morphogenesis have been identified. (5) A cis-acting regulatory sequence that may be necessary for the expression of a cuticle protein gene may have been identified by "footprinting" experiments.

Publications: 88/01 to 88/12

KISS, I., BEATON, A., TARDIFF, J., FRISTROM, D. & FRISTROM, J. (1988). Interactions and developmental effects of mutations at the Broad-Complex of Drosophila melanogaster. Genetics, 118, 247-259.

KIMBRELL, D., KING, D.S., WOLFGANG, W., & FRISTROM, J.W. (1988). Cuticle protein gene expression during the third instar of Drosophila melanogaster. Insect Biochem., 18, 229-235.

BEATON, A., KISS, I., & FRISTROM, J.W. (1988). Interactions between the Broad-Complex and the Stubble-stubbloid locus in Drosophila. Genetics, 120: 453-464.

NATZLE, J., FRISTROM, D., & FRISTROM, J.W. (1988). Genes expressed during imaginal disc morphogenesis. IMP-E1, a gene implicated in epithelial cell rearrangement. Dev. Biol. 129: 428-438.

OSTERBUR, D., NATZLE, J., FRISTROM, D., TOJO, S.J., & FRISTROM, J. (1988). Genes expressed during imaginal disc morphogenesis. IMP-L2, a gene implicated in epithelial fusion. Dev. Biol. 129: 428-438.

FECHTEL, K., NATZLE, J., BROWN, E., & FRISTROM, J. (1988). Prepupal differentiation of Drosophila imaginal discs: Identification of four genes whose transcripts accumulate in response to a pulse of 20-hydroxyecdysone. Genetics, 120:.

FRISTROM, D., (1988). The cellular basis of epithelial morphogenesis. A review.

65.006 CRIS0136124

MOLECULAR APPROACH TO A GENE CONFERRING NEMATODE RESISTANCE TO TOMATO

WILLIAMSON V M; Nematology; University of California, Davis, **CALIFORNIA** 95616.
Proj. No.: CA-D*-NEM-5001-CG Project Type: CRGO Agency ID: CRGO Period: 01 JUL 88 to 30 JUN 91

Objectives: PROJ. 8800667. Mi is a dominant locus that confers resistance to root-knot nematodes when present in tomato. Our goal is to clone Mi to increase understanding of the mechanism of resistance conferred by this gene.

Approach: Mi is closely linked to Aps-1, encoding acid phosphatase-1 in tomato. Acid phosphatase-1 will be purified from cell suspension culture and used to produce antibody and obtain peptide sequence for identification of the corresponding cDNA. Using this clone and clones of other linked fragments (RFLPs) as probes, DNA from resistant and susceptible cultivars will be analyzed. Candidate clones of Mi will be obtained by "chromosome walking" techniques. We propose to identify Mi by complementation of function after transformation of susceptible tomato cultivars with candidate clones using Agrobacterium based vectors.

Progress: 88/08 to 88/12. We have obtained data that will be of value in our attempt to clone the nematode resistance gene Mi of tomato by chromosome walking from the linked gene Aps-1. Using DNA clones which flank the region of Chromosome VI, which carries Aps-1 and Mi, 7 and 11 genetic map units away, we examined DNA from various tomato cultivars. Southern blot analyses were carried out using DNA from tomato cultivars that differ in the Aps-1 alleles, and in whether they carried Mi. Our results indicate that the size of the region of the tomato genome derived from the wild tomato species L. peruvainum (the source of Mi) varies among cultivars. This region of DNA is quite extensive in some cultivars, where it includes a DNA marker that is 7 map units away from Aps-1. This information will help us to localize the region of DNA encoding Mi for our chromosome walking experiments.

Publications: 88/08 to 88/12

No publications reported this period.

65.007 CRIS0096636

MOLECULAR DIAGNOSTICS OF PLANT PARASITIC NEMATODES

PLATZER E G; Nematology; University of California, Riverside, **CALIFORNIA** 92521.
Proj. No.: CA-R*-NEM-4575-CG Project Type: CRGO Agency ID: CRGO Period: 01 SEP 85 to 31 AUG 90

Objectives: Proj. 8500495. To develop recombinant DNA techniques for detection of host-races of root-knot nematodes (Meloidogyne spp.). Such studies will facilitate selection of appropriate resistant cultivars, crop rotation, or biological contron in crop management strategies.

Approach: Mitochondrial DNA will be isolated from populations of root-knot nematode species and host-races. The mt DNA will be characterized physically by fine structure restriction mapping. Libraries of recombinant DNA hybrid plasmid molecules containing mt DNA from host-races will be constructed, screened for use in diagnostic molecular probes and assessed for sensitivity to nematode lysates from soil extracts.

Progress: 88/01 to 88/12. During investigations of a Southern California, San Bernardino Co. root-knot nematode population identified as Meloidogyne hapla (M. hapla BRDO strain), heterogeneity in CsC1-gradient-purified mitochondrial DNA (mtDNA) was discovered. Gradient purified mtDNA from cultures of this population, when digested with Hind III and the restriction fragments separated on agarose gels, results in two sets of restriction fragments with different ethidium bromide fluorescent stain intensities. Southern Blots of gels used to separate restriction digests of M. hapla and Meloidogyne chitwoodi mtDNA were probed with a cloned mitochondrial gene (co-1 from Romanomermis culici-vorax. Hybridization of the co-1 probe to specific Hind III restriction fragments demonstrated that both the intense and the fainter sets of fragments were mtDNA. This has also enabled assignment of co-1 to specific restriction fragments of both M. chitwoodi and M. hapla. Further studies on populations derived from single egg masses showed that the restriction endonuclease fragment patterns

segregated and were independent. Thus at least two varieties of M. hapla can be distinguished by characterizing mtDNA.

Publications: 88/01 to 88/12
No publications reported this period.

65.008 CRIS0136380
GENETICS OF LYMANTRIA DISPAR AND ITS BACULOVIRUS

KNUDSON D L; Colorado State University, Fort Collins, **COLORADO** 80523.
Proj. No.: COLR-8802297 Project Type: CRGO
Agency ID: CRGO Period: 01 SEP 88 to 31 AUG 90

Objectives: PROJ. 8802297. The overall objective of this proposal is to examine this interaction at a genetic level using the gypsy moth, Lymantria dispar, and its viral system, L. dispar multiple-embedded nuclear polyhedrosis virus (LdMNPV); with the goal of using this knowledge to improve the potency and efficacy of a baculovirus pesticide.

Approach: The experimental approaches will include the construction of a genomic library of LdMNPV which will be used to generate a physical map of LdMNPV and its genomic variants. Regions on the genome where variation occurs will be identified. A genomic library of the host will be constructed and used in the identification of host cell inserts in the viral genome. The cellular DNA inserts will be characterized to determine whether they are expressed in uninfected cells. The cellular inserts will be sequenced, and their copy number in L. dispar will be determined. The viral insertional regions will be examined to determine the effect that the insertion of host cell DNA has on viral transcription, and these results will be compared with transcription in virulent wild-type virus infections in vitro.

65.009 CRIS0034048
GENETICS OF GYPSY MOTH POPULATIONS

HARRISON R G; Biology; Yale University, New Haven, **CONNECTICUT** 06520.
Proj. No.: 8300098 Project Type: CRGO
Agency ID: CRGO Period: 01 JUL 83 to 30 JUN 85

Objectives: Project Number 8300098. The objectives of this research are to define genetic relationships among populations of the gypsy moth from throughout its range and to understand the genetic consequences for the gypsy moth of its introduction into the United States.

Approach: We will use data on genetic similarity to reconstruct the recent history of the moth and to identify probable source area(s) in Eastern Asia for the moths that have spread across Eurasia. We will use similar information to determine whether there exist genetic differences among gypsy moth populations in the United States. Estimates of genetic similarity will be based on examination of biochemical markers and on quantitative genetic analysis of life history characters.

Progress: 86/01 to 86/12. During the course of this project, we: isolated and purified mitochondrial DNA (mtDNA) from gypsy moths; constructed a restriction site map of mtDNA for a laboratory strain; initiated a study of genetic affinties among gypsy moths from Asia, Europe, and North America; and carried out an experiment to determine the extent of genetic variation and differentiation in larval development within and among North American populations. We now have a proven methodology for preparation of large amounts of mtDNA from eggs of virgin females. Nick-translation of this pure mtDNa provides us with a P-labeled probe to hybridize with restriction enzyme digests of total DNA from individual moths. In this way, we have examined restriction site variation among moths from North America, France, China and Japan. There is no variation within or among N.A. populations and these individuals are identical in their restriction map to moths from South France. However, there are a considerable number of site differences between the N.A./European moths and those from Asia. The results suggest that it may well be possible to identify Asian populations that have been "source" populations for the spread of the gypsy moth to Europe and N.A. Additional material from Asia is needed, but we were constrained to use moths already in our freezer because of the absence of a local quarantine facility (which we expected to have completed before termination of this project).

Publications: 86/01 to 86/12
NO PUBLICATIONS REPORTED THIS PERIOD.

65.010 CRIS0034218
EUROPEAN CORN BORER PHEROMONE STRAINS: FATE OF HYBRIDIZING POPULATIONS

HARRISON R G; Biology; Yale University, New Haven, **CONNECTICUT** 06520.
Proj. No.: CONR-8400258 Project Type: CRGO
Agency ID: CRGO Period: 13 JUL 84 to 31 JUL 86

Objectives: PROJ 8400258. The objectives of this research are to understand genetic and evolutionary relationships between two "pheromone strains" of the European Corn Borer. We wish to establish the degree of reproductive isolation (or conversely, the extent of hybridization) between the two strains - where they occur together.

Approach: We will first identify appropriate biochemical markers (variation in enzyme mobility on gels and variation in restriction fragment patterns of mitochondrial DNA) and then use these markers to analyze hybridization and introgression in field populations. This work will be carried out in conjunction with studies of pheromone production and response and mating behavior (carried out by R.T. Carde).

Progress: 86/01 to 86/12. We have focused our research effort on developing biochemical genetic markers for analyzing hybridization and introgression between European Corn Borer (ECB) pheromone strains. The major thrust of the work to date has been (1) to isolate and purify mitochondrial DNA (mtDNA) from a single laboratory strain (a (Z)-strain from Iowa being reared in Ring Carde's laboratory at University of Massachusetts), and (2) to establish a restriction site map for ECB mtDNA. Isolation and purification of mtDNA proved more difficult than we expected, primarily because of the need for very large numbers of moths in each preparation. However, using standard techniques of differential centrifugation and CsC1 equilibrium density gradient centrifugation, we have now successfully prepared ECB mtDNA from two sources: (1) thorax (males and females combined); (2) abdomen (virgin females). This purified mtDNA has been nick-translated and used as a probe to hybridize with restriction endonuclease digests of total DNA from individual moths. Resulting autoradiographs are very clear and allow us to map restriction sites. Using standard double-digest procedures we have mapped sites for eleven of these enzymes (total of 23 sites). Additional sites will be mapped as soon as a laboratory is available at Cornell. We are thus poised to (1) document mtDNA restriction site differences between the two pheromone strains (if they exist) and (2) examine mtDNA composite genotypes in individual moths from mixed populations.

Publications: 86/01 to 86/12
NO PUBLICATIONS REPORTED THIS PERIOD.

65.011 CRIS0047763
GENETICS OF STERILITY IN HELIOTHIS

MILLER S G; Agricultural Research Service; Agricultural Research Service, Gainesville, **FLORIDA** 32601.
Proj. No.: 6615-20250-018-00D
Project Type: INHOUSE
Agency ID: ARS Period: 02 JUL 82 to 12 FEB 87

Objectives: Compare the structure of the mitochondrial DNA of various species of Heliothis. Investigate the interactions between the nuclear and mitochondrial genomes during spermatogenesis, the role of these interactions in inherited male sterility, and whether such sterility can be induced by genetic engineering methods.

Approach: Electrophoresis of the restriction enzyme digestion products of mitrochondrial DNA will be used to develop detailed comparative maps of the genomes of various species and, along with other methods, to estimate H. virescens; hybrid ratios. Basic cytological, biochemical and molecular genetic studies will be conducted on the role of the mitochondrion in spermatogenesis. The genetics of mitrochondrial membrane proteins will be investigated using the Heliothis hybrid system in order to identify aberrations in normal protein interactions. The molecular genetic basis of such aberrations will be elucidated and methods for the induction of such aberrations in other species will be investigated. e.

Progress: 86/01 to 86/12. We have conducted experiments designed to elucidate the mechanism of male sterility in hybrid males produced from interspecific matings between the male tobacco budworms, Heliothis virescens, and H. subflexa females. The DNA sequences corresponding to a number of mitochondrial genes have been cloned and characterized. These detailed studies have revealed that the function of these genes is impaired in hybrid males and may account for their sterility. The results suggest that other insect pests can be sterilized by introducing altered versions of these genes into their chromosomes.

Publications: 86/01 to 86/12
NO PUBLICATIONS REPORTED THIS PERIOD.

65.012* CRIS0138045
IDENTIFICATION, BEHAVIORAL ECOLOGY, GENETICS AND MANAGEMENT OF AFRICAN HONEYBEES

HALL H G; Entomology & Nematology U.s. Horticultural Lab; University of Florida, Gainesville, **FLORIDA** 32611.
Proj. No.: FLA-ENY-02791 Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 88 to 30 SEP 93

Objectives: Develop and improve techniques for identifying individual worker, queen and drone Africanized honeybees (AHBs). Monitor the entry and spread of the AHB genotype into the U.S., and determine contributions of swarming and mating to AHB spread. Before and during African honeybee migration, bee samples will be collected from regions of northern Mexico, Texas, Louisiana, Mississippi, Arizona, California and Florida. The samples will be obtained from trapped drones, feral swarms and established apiaries spatially arranged as transects of grids.

Approach: DNA restriction fragment length polymorphisms (RFLPs) will be sought that can identify African and European honeybees. Samples will be tested for nuclear and mitochondrial DNA RFLPs already characterized and for new nuclear DNA RFLPs expected to be found. Spatial and temporal spread of nuclear and mitochondrial markers will distinguish African bee paternal and maternal gene flow into the extant European populations. Distribution patterns of genotypes within the temperate United States may point to processes affecting hybridization between African and European honeybees.

65.013 CRIS0048579
MOLECULAR GENETICS OF SPERMATOGENESIS AND STERILITY IN HELIOTHIS

YU S S J; HUETTEL M D; Inst of Food & Agri Sciences; University of Florida, Gainesville, **FLORIDA** 32611.
Proj. No.: 6615-22240-001-01S
Project Type: COOPERATIVE AGREE.
Agency ID: ARS Period: 30 SEP 83 to 30 SEP 88

Objectives: Understand the physical structure of the mitochondrial (mt) genome and patterns of transcription and translation of both mt and nuclear genes encoding sperm mt proteins. Determine the precise subcellular and suborganellar distribution of these proteins. Develop means for introducing DNA into embryos and cultured cells. Apply this knowledge to understanding the mechanism of male sterility in Heliothis backcross hybrids..

Approach: Differences observed in the structure of mtDNA, or the transcription and translation processes of mt and nuclear genes active in mt function during spermatogenesis in H. virescens and backcross hybrids will be studied using electron microscopy, comparative restriction maps and clones available in our library of testes-active genes. Antibodies to species-specific sperm mt proteins will be used for indirect immunofluorescence microscopy to localize the relevant proteins within the developing sperm cell mitochondria. Identified genes linked to transposable elements and mtDNA molecules will be microinjected into cultured cells and embryos to duplicate observed genetic lesions in sterile males. Techniques developed will be applied to the induction of sterility in other insect species..

Progress: 88/01 to 88/12. A cDNA clone corresponding to the "p63" protein (a polypeptide suspected of being dysfunctional in H. virescens x H. subflexa backcross hybrids) was isolated from an expression library using antiserum. Subsequent sequencing of this DNA revealed 60% homology at the nucleotide level, and greater than 70% homology at the amino acid level, to groE heat shock proteins. Consistent with this observation, we have found that the p63 polypeptide shares several properties with groE proteins including ATPase activity, affinity for other mitochondrial proteins, and a characteristic ring-shaped structure comprised of seven subunits. The well characterized role served by groE proteins from other organisms in assembling protein complexes has suggested numerous experimental approaches toward determining the role served by p63 in spermiogenesis. A full-length copy of the p63 gene has also been recovered from a genomic library and is in the process of being characterized more completely. The availability of this clone will permit transformation-based studies of the regulation and processing of the p63 polypeptide to proceed.

Publications: 88/01 to 88/12
NO PUBLICATIONS REPORTED THIS PERIOD.

65.014 CRIS0133749
PURIFICATION OF A NEUROPEPTIDE HORMONE FROM MOSQUITOES: A POSSIBLE MODEL FOR NOVEL PESTICIDES

LEA A O; BROWN M R; Entomology; University of Georgia, Athens, **GEORGIA** 30602.
Proj. No.: GE000948 Project Type: HATCH
Agency ID: CSRS Period: 01 JAN 88 to 31 DEC 91

Objectives: We propose to develop in vitro and biological assays to guide the purification of prothoracicotropic hormone (PTTH) from mosquito larvae. If we obtain a partial sequence, we will isolate and clone the gene for PTTH.

Approach: We will use conventional chromatography and HPLC to isolate PTTH for sequencing. Sequencing will be carried out at the University of Georgia Genetics Sequencing Laboratory and in collaboration with Drs. A. Suzuki and Shogo Matsumoto, Department of Biochemistry, University of Tokyo. Peptide sequences will be synthesized and used in physiological tests and for construction of oligonucleotide probes to identify the PTTH gene(s).

Progress: 88/01 to 88/12. We have been developing both in vivo and in vitro bioassay systems to test fractions from the various purification steps being followed in our attempt to isolate prothoracicotropic hormone (PTTH) from heads of fourth instar Aedes aegypti larvae. The in vitro system utilizes prothoracic segments incubated with test fractions, and a RIA for ecdysteroides in the medium. This is a time consuming assay and is only used to verify the positive results from in vivo experiments. We have been working to increase the sensitivity of the in vivo assay and have found that by feeding the larvae six hours before injecting the test extracts, the assay is significantly improved. Two sets of fractions from the firstchromatographic step appear to contain most of the activity, suggesting that PTTH comes in at least two molecular classes. Both the in vivo and the in vitro assays are being repeated before preceding to the next purification step. Production of fourth instar larvae continues in order to accumulate a supply of heads for future purification. The firs extractions were made from approximately 550,000 heads. Even with a mass rearing facility that produces 250,000 females per week, the small quantity of hormone in each individual and the large losses incurred during the 7-8 purification steps, indicate that this will be a long-term project.

Publications: 88/01 to 88/12
NO PUBLICATIONS REPORTED THIS PERIOD.

65.015 CRIS0130942
IMPROVING THE EFFICACY OF BACULOVIRUS PESTICIDES BY RECOMBINANT DNA TECHNOLOGY

MILLER L K; Entomology; University of Georgia, Athens, **GEORGIA** 30602.
Proj. No.: GEO-RC293-110 Project Type: CRGO
Agency ID: CRGO Period: 01 OCT 86 to 30 SEP 87

Objectives: PROJECT 8603163. Improve the efficacy of insect baculoviruses as biological pesticides by introducing insect behavior-modifying genes into baculoviruses. Use recombinant DNA technology to genetically construct a baculovirus that expresses a foreign gene which affects insect behavior.

Approach: Construct recombinant baculoviruses that carry a gene encoding an insect-specific neurotoxin and produce large quantities of toxin in infected insect cells. Determine if the recombinant virus is a more effective biological pesticide and if it has an extended host-range. Study possible strategies for reducing recombinant virus persistance in the environment to enhance the ecological safety of the pesticides.

Progress: 86/10 to 87/09. A gene encoding an insect-specific neurotoxin of scorpion venom, the Buthus eupeus insectotoxin I gene (BeIt1), has been synthesized from oligoneucleotides based on the published amino acid sequence of the toxin. The synthetic gene was cloned in E. coli and the sequence of the gene was confirmed by DNA sequencing. The BeIt1 gene was then transferred to an E. coli gene expression vector utilizing the Tac promoter to drive expression. No toxin expression was observed above background endotoxin activity in E. coli. The BeIt1 gene was transferred to a baculovirus expression system utilizing the polyhedrin promoter to drive toxin expression. No toxin activity was observed in recombinant BeIT1 baculovirus-infected cells. Toxin gene expression in infected insect cells was monitored at the protein synthesis level by pulse-labeling proteins with radioactive methionine. Only very low levels of a new 3.7 kilodalton protein was observed in the recombinant virus-infected cells. It is likely that the BeIT1 toxin is highly unstable under the conditions of expression used and if this gene is to be used to enhance baculovirus pesticide efficacy, a means of stabilizing the small polypeptide will need to be developed. The safety of recombinant baculoviruses with respect to mammalian species was also investigated during the course of this work.

Publications: 86/10 to 87/09

CARBONELL, L.F. and MILLER, L.K. 1987. Appl. Environ. Microbioly, 53:1412-1417. Baculovirus interaction with nontarget organisms: A virus-borne reporter gene is not expressed in two mammalian cell lines.

CARBONELL, L.F. and MILLER, L.K. 1987. Genetic engineering of viral pesticides: Expression of foreign genes in nonpermissive cells. In "Molecular Strategies for Crop Protection".

MILLER, L.K. 1987. Expression of foreign genes in insect cells. In "Biotechnology Advances in Invertebrate Pathology and Cell Culture" (ed. K. Maramorosch). Academic Press, Orlando, Fl.

65.016 CRIS0134015

REGULATION OF EXPRESSION OF THE BACULOVIRUS, ACNPV

FRIESEN P D; Bacteriology & Biochemistry; University of Idaho, Moscow, **IDAHO** 83843.
Proj. No.: IDA00908 Project Type: HATCH
Agency ID: CSRS Period: 01 JAN 88 to 06 JAN 89

Objectives: The long term objective of this proposal is to genetically engineer the baculoviruses for improved efficacy as biological control agents of insect pests. Our immediate goals are to: determine the organization of several early viral genes for the insertion of foreign genes, locate those DNA sequences responsible for early and regulated expression of such genes, and examine the function of the early genes determing whether they are nonessential and are therefore replaceable. This proposal also examines the nature and mutagenic effects of an insect-derived transposable element which has integrated into the DNA genome of the baculovirus, AcNPV. Transposable elements act to decrease the virus' ability to produce its occluded form thereby reducing viral pesticide effectiveness.

Approach: Viral gene organization and the location of DNA control regions will be analyzed by fusing specific genes to easily assayed reported genes and testing them for proper expression in transient assays and by placing them back into the viral genome. Antibodies raised to trihybrid viral fusion proteins will be used to examine viral gene function. The gene organization and mutagenic effects of the transposable element will be determined by DNA sequence analysis and nucleic acid hybridization techniques.

Progress: 88/01 to 88/12. Progress in our investigation of the molecular mechanisms involved in the regulation of baculovirus gene expression has been the identification of DNA sequences responsible for conferring early and late transcription of the gene encoding a 35,000 - molecular - weight protein (35K) in the HindIII-K genome region of Autographa californica nuclear polyhedrosis virus (AcNPV). Mutagenesis of the 35K gene promoter previously linked to the reporter gene for chloramphenicol acetyl-transferase, indicated that sequences from -155 to -55 relative to the RNA start site (position +I) controlled early transcription while sequences from -55 to -4 controlled late transcription in recombinant viruses. Thus, two distinct regions of the 35K gene promoter are involved in early versus late regulation. Both regions contain sequences found at the promoter of other AcNPV genes with similar regulation and may therefore represent common control sequences. These studies on the nature of AcNPV promoters provide necessary information for the construction of recombinant baculoviruses expressing insecticidal genes for improved biological control of insect pests.

Publications: 88/01 to 88/12

NISSEN, M.S., and FRIESEN, P.D., (1989). Molecular Analysis of the Transcriptional Regulatory Region of Early Baculovirus Gene. J. Virology (in press).

65.017 CRIS0083485
ORGANIZATION AND EXPRESSION OF A BACULOVIRUS DNA GENOME

MILLER L K; Bacteriology & Biochemistry; University of Idaho, Moscow, **IDAHO** 83843.
Proj. No.: IDA00801 Project Type: HATCH
Agency ID: CSRS Period: 01 JUL 84 to 30 JUN 89

Objectives: The long-term objectives of this proposal are to improve the efficacy of viral pesticides by genetic engineering technology and facilitate the commercial production of these viruses. To achieve these objectives, more information conce ning viral gene organization and the regulation of gene expression is required. The immediate goals are, therefore, to locate key viral genes with respect to a physical map of the DNA genome, test an early promoter for controlling early expression of a passenger gene, and define the nature of genes involved in controlling gene expression. Since a handicap in the commercial production of viruses in cell culture was traced to the insertion of mobile genetic elements into the viral DNA.

Approach: Key genes of the baculovirus AcNPV will be mapped with respect to the established physical map of the viral DNA by marker rescue and by cloning cDNAs of early and intermediate viral mRNAs. Detailed knowledge of the nature of one such gene will be obtained; its promoter will be fused to an easily assayable gene and tested for temporal regulation. The position preferences of transposable element insertions will be determined and their effect on viral gene expression will be determined by nucleic acid hybridization techniques.

Progress: 86/01 to 86/12. Research has progressed in several different areas relevant to baculovirus gene orgainzation and expression that are applicable to development of more effective viral pesticides. First, we have synthesized and cloned complementary DNA from 20 different regions of the viral genome. Temporal expression of RNA from each region was examined and all were found to contain overlapping sets of RNA. Many of these overlapping sets of RNA have common 5' or common 3' termini, a common motif in the organization and expression of baculovirus genes. These studies are important since an understanding of the regulation of viral expression is required before the virus can be successfully engineered (via recombinant DNA) as improved pesticides. Secondly, to better understand this regulation, we have analyzed the structure of viral DNA during the viral replication cycle. We found that the viral genome adapts a nucleosomal-like structure typical of DNA undergoing active transcription. Thirdly, we have continued studies on the molecular biology of a transposable element (TED) which inserted into the baculovirus genome causing mutations. This led to the discovery that TED is a member of a newly characterized class of mutagenic elements which resemble the RNA tumor viruses.

Publications: 86/01 to 86/12

WILSON, J. and MILLER, L.K. 1986. Changes in the nucleoprotein complexes of a baculovirus DNA during infection. Virology 151:315-328.
MAINPRIZE, T.M., LEE, K.-J. and MILLER, L.K. 1986. Variation in temporal expression of overlapping baculovirus transcripts. Virus Res. 6:85-89.
FRIESEN, P.D., RICE, W.C., MILLER, D.W. and MILLER, L.K. 1986. Bidirectional transcription from a solo long terminal repeat of the retrotransposon Ted: Symmetrical RNA start sites. Mol. Cell. Biol. 6:1599-1607.

65.018 CRIS0136883
STUDIES OF BACILLUS THURINGENSIS PROTOXINS OVERCOMING INSECT RESISTANCE

ARONSON A I; DUNN P E; JOHNSON D E; Division of Sponsored Programs; Purdue Res Foundation, West Lafayette, **INDIANA** 47907.
Proj. No.: INDR-8800791 Project Type: CRGO
Agency ID: CRGO Period: 01 SEP 88 to 31 AUG 89

Objectives: PROJ. 8800791. Characterize two protoxins in a B.t. subspecies aizawai isolate which kills resistant Plodia. The genes for these protoxins will be cloned and sequenced and the effectiveness of the protoxins will be examined alone and in concert. Other protoxin genes with a chromosomal rather than a plasmid location will be studied for expression and possible activation. An enrichment for Plodia resistant to other B.t. subspecies will be attempted. Vesicles will be prepared from the midguts of resistant and sensitive Plodia and used to develop an assay for the effectiveness of various B.t. toxins.

Approach: Clones of plasmid or total DNA from B.t. subspecies aizawai will be screened with several protoxin gene probes. The cloned genes will be transformed into E. coli, for expression and bioassays on resistant and sensitive Plodia. Studies of chromosomal genes will involve Southern hybridization to compare gene locations. Colonies of Plodia will be reared on diets containing various B.t. preparations in order to select for resistance. Vesicles will be assayed by measuring potassium-dependent amino acid transport in the presence or absence of various toxins.

65.019* CRIS0074454
DEFENSIVE RESPONSES IN INSECTS AND PLANTS

DUNN P E; Entomology; Purdue University, West Lafayette, **INDIANA** 47907.
Proj. No.: IND058047 Project Type: HATCH
Agency ID: CSRS Period: 15 NOV 88 to 30 SEP 93

Objectives: Analysis of the structure, synthesis, and function of hemofibrin. Characterization of peptidoglycan regulated protein synthesis and feeding inhibition. Determination of the structure of antibacterial protein genes and characterization of

mechanisms regulating their expression. Characterize antibacterial protein synthesis by Malpighian tubules. Analysis of the mechanism of parasitoid-induced hemocyte dysfunction. Transformation of a plant with CLP gene to confer increased resistance to bacterial pathogens.

Approach: Protein structural analysis, molecular cloning, nucleotide sequence analysis, quantitative binding assays, analysis of second messenger titer and metabolism, monitoring bio-activity, in vitro transcription run-off assays, in vitro organ culture, in situ hybridization, immunocytochemical analysis, plant transformation, Northern hybridization analysis, assay of susceptibility of transformed plants to bacterial pathogens.

Progress: 87/10 to 88/09. We have identified a 22kD protein, hemofibrin, which is a constitutive component of the hemolymph of immunologically naive larvae of Manduca sexta. When hemolymph contacts wounded tissue surfaces, hemofibrin rapidly aggregates to form a fibrous coagulum like that formed from the vertebrate protein fibrin during blood clotting. The fibrous coagulum formed from hemolymph hemofibrin serves as a net and traps hemocytes present in the hemolymph which flows from the wound. The resulting hemocyte/hemofibrin coagulum appears to form the primary clot which plugs wound openings. Hemofibrin has been purified in native (unassembled) form hemolymph of M. sexta. Analysis of hemofibrin primary structure and of the aggregation process are in progress. Previous results from RNase protection experiments demonstrated that treatment of M. sexta larvae with peptidoglycan induced lysozyme, cecropin-like peptide, and attacin-like protein (ALP) genes in Malpighian tubules, resulting an accumulation of RNA transcripts from these genes. Using in vitro culture techniques, we have confirmed the peptidoglycan-regulated synthesis and secretion of lysozyme and ALP proteins by Malpighian tubule tissue. Studies of the suppressed antibacterial response in M. sexta parasitized by Cotesia congregata have demonstrated that a functional hemocyte-mediated response is not required for the development or maintenance of an "immunized" state after injection of bacteria or peptidoglycan.

Publications: 87/10 to 88/09

DAI, W. (1988). Structure and regulation of the synthesis and secretion of an attacin-like protein from larvae of Manduca sexta. Ph.D. Thesis, Purdue University, W. Lafayette, IN.

ROSS, D. R. (1988). Effects of parasitism by Cotesia congregata on the antibacterial responses of the tobacco hornworm, Manduca sexta. Ph.D. Thesis, Purdue University, W. Lafayette, IN.

KANOST, M. R., W. DAI, and P. E. DUNN. (1988). Peptidoglycan fragments elicit antibacterial protein synthesis in larvae of Manduca sexta. Arch Insect Biochem. Physiol. 8:147-164.

GENG, C. and P. E. DUNN. (1988). Hemostasis in larvae of Manduca sexta: formation of a fibrous coagulum by hemolymph proteins. Biochem. Biophys. Res. Comm. 155:1060-1065.

DICKINSON, L. V. RUSSELL and P. E. DUNN. (1988). A Family of bacteria-regulated, cecropin D-like peptides from Manduca sexta. J. Biol. Chem., in press.

65.020 CRIS0096260

GENETIC ANALYSES OF PATTERN FORMATION AND REGULATION IN DROSOPHILA

GIRTON J R; Genetics; Iowa State University, Ames, **IOWA** 50011.
Proj. No.: IOW02746 Project Type: STATE
Agency ID: SAES Period: 01 JUL 85 to 30 JUN 90

Objectives: To define the nature of the genetic function of genes responsible for developmental control mechanisms. Alleles at the suppressor-of-forked locus cause major changes in limb and head development in Drosophila. We will study their mechanisms of action.

Approach: We will isolate several new mutations using a transposable element mutagenesis screen, and will compare their developmental effects with known suppressor-of-forked mutations. We will isolate and clone the DNA corresponding to the normal form of the suppressor-of-forked gene and will compare this with DNA from mutant forms. This will give information about the location of genetic functional sites within the gene.

Progress: 88/01 to 88/12. We are investigating three genes that regulate growth and development in Drosophila: Pleiohomeotic, a homeotic gene that controls cell determination in the head and legs, suppressor of forked a complex gene that regulates transcription of specific genes, and Blackpatch, a gene we discovered that regulates neural development in the optic lobe of the brain. This year, new studies of the interactions of mutations of Pleiohomeotic with other homeotic mutations were begun. These studies will define how this gene fits in the homeotic gene networks that control cell determination during development. The first mutations being tested are ash-1 and ash-2, provided by Dr. Allen Shearn at Johns Hopkins and Polycomb-3, provided by Dr. Robin Denell at Kansas State. Our study of suppressor of forked, su(f) is nearly completed. Northern analyses of the target gene sgs-3 done this past year confirm that su(f) contains distinct suppression and lethal domains. Our analysis of the neural development gene Blackpatch has expanded. Eight new alleles have been discovered and the gene has been mapped to position 71.8 on the third chromosome. One additional mapping study is in progress to confirm this map position. Morphological analyses indicate Blackpatch mutations alter or block the development of the optic lobes of the adult brain and interact with some facet alleles of the Notch locus.

Publications: 88/01 to 88/12

No publications reported this period.

65.021 CRIS0136888
INSECT CONTROL BY MANIPULATION OF CUTICLE DEGRADING ENZYMES AND THEIR GENES

KRAMER K J; MUTHUKRISHNAN S; Us Grain Marketing Res Lab; 1515 College Ave, Manhattan, **KANSAS** 66502.
Proj. No.: KANR-8800568 Project Type: CRGO
Agency ID: CRGO Period: 01 JUL 88 to 30 JUN 91

Objectives: PROJ. 8800568. The genetic regulation of cuticle degradation in molting insects will be investigated at the molecular level with the resulting information used to design and synthesize compounds that adversely affect the molting process.

Approach: The structure and expression of insect cuticle degrading enzyme genes will be investigated at the molecular level to obtain information about the genetic manipulation of those genes for insect control purposes. A library of epidermal cDNA clones will be prepared that contains sequences coding for molting hormone inducible cuticle hydrolytic enzymes. Enzyme probes will be prepared from clones characterized by hybrid selection and immunoprecipitation using antibodies specific for molting enzymes. Molting enzyme cDNA will be subcloned for DNA sequence analysis and used to probe a genomic library. Regions of genomic clones containing coding and regulatory sequences will be identified and characterized.

65.022* CRIS0049420
MOLECULAR AND BIOCHEMICAL GENETICS OF TRICHOGRAMMA AND HELIOTHIS FOR BIOSYSTEMATICS AND BIOCONTROL

HUETTEL M D; HUNG A C F; Beneficial Insect Intro Lab Insect Iden & Bene Insect Inst; Beltsville Agr Res Center, Beltsville, **MARYLAND** 20705.
Proj. No.: 1275-21240-001-00D
Project Type: INHOUSE
Agency ID: ARS Period: 01 OCT 85 to 30 SEP 90

Objectives: Develop and adapt molecular, biochemical, and other genetic technologies for the systematics and identification of Trichogramma and Heliothis spe- cies and to the biological control of Heliothis.

Approach: Characterize populations and species of Trichogramma parasitoids and Helio-this moths using isozyme analyses, genetic maps of mitochondrial DNA (mtDNA), and advanced chromosomal techniques as appropriate. Define specieslimits within Trichogramma by cross-mating experiments. Investigate methodsfor the induction of mitochondrially-based inherited male sterility in H. zea. Augment control of H. virescens by conferring virus resistance upon released male-sterile backcross hybrids. Develop monoclonal antibodies against insect extracts for use in ELISA and RIA. Beltsville, MD, Bg 225; BL-1; approval request in preparation. Scientists and technicians associated with project: M. Huettel, E. Quinn, W. Sheppard.

Progress: 88/01 to 88/12. The development an of ELISA kit for field identification of Africanized honey bees is experiencing expected difficulties, because subspecies-specific antigens are not yet available. Different methods of immunosuppression are being tried to overcome these difficulties. The first case of parasitic hymenoptera culture changing from arrhenotoky to thelytokoy was documented with evidence from analysis of 23 isozyme loci. The MS was submitted for peer review. Developmental stage-specific isozyme loci were identified in the marsh fly, Sepedon fuscipennis. The information is essential for the biosystematic study in the S. fuscipennis species complex. A total of 24 lots of Trichogrammatidae from 12 states and 3 countries were received and identified. Progress has been made in adapting techniques for extraction of mtDNA from single, frozen heliothine adults for species' comparisons using Southern blots of mtRFLPs visualized by nonradioactive probes. Small sample sizes of rare species continue to be a problem, however. The genetic relationships between previously described genes controlling pheromone production in females and response in males of the European corn borer were described in field and lab studies. A rapid molecular technique for identification of Africanized honey bees using restriction enzyme analysis of mitochondrial DNA was developed.

Publications: 88/01 to 88/12

HUNG, A.C.F., DAY, W.H. and HEDLUND, R.C. 1988. Genetic variability in arrhe notokous and thelytokous forms of Mesochorus nigripes (Hym.:Ichneumonidae) . Entomophaga 33: 7-15.

HUNG, A.C.F. 1988. Taxonomic treatment of thelytokous forms in parasitic Hymenop tera. In: V.K. Gupta, ed., Advances in Parasitic Hymenoptera Research, pp. 163-167.

KLUN, J.A. and HUETTEL, M.D. 1988. Genetic regulation of sexpheromone production and response: Interaction of sympatric types of European corn borer, Ostrinia n ubilalis (Lepidoptera:Pyralidae). Jour. Chem. Ecol. 14:2047-2061.

LASTER, M., CARPENTER, J., HUETTEL, M., et al. 1988. Auto- cidal systems for He liothis control. In: Herzog, S. et al., eds., Theory & tactics of Heliothis PopM anag.:III. Emerging control tactics & tech. S. Coop. Ser Bull. 337:74-103.

SHEPPARD, W.S. and HUETTEL, M.D. 1988. Biochemical genetic markers, intraspecif ic variation, & population genetics of the honey bee, Apis mellifera. In: Needh am et al. eds., Africanized Honey Bees & Bee Mites, E. Horwood Ltd, Eng.

65.023* CRIS0048495
ARTHROPOD VIRUSES: CHARACTERIZATION, GENETICS AND REPLICATION IN VIVO AND IN VITRO

VAUGHN J L; TOMPKINS G J; ADAMS J R; Insect Pathology Lab Plant Protection Inst; Beltsville Agr Res Center, Beltsville, **MARYLAND** 20705.
Proj. No.: 1275-22240-002-00D
Project Type: INHOUSE
Agency ID: ARS Period: 29 JUN 83 to 29 JUN 88

Objectives: To characterize viruses from insect pests and evaluate their potential for use in pest management. To determine biochemical processes of virus invasion, replication and virulence and the genetic factors controlling these processes. To develop the necessary in vitro systems for the isolation, replication and production of insect pathogenic viruses.

Approach: Characterization will be done by a combination of light and electron microscope studies and by biochemical analysis of the structural components of the virus. Genetic relatedness to other viral isolates from the same or similar insect species will be determined by restriction endonuclease analysis of the viral genome and by nucleic hybridization tests. Genetic manipulation will be either by classical methods of viral recombination or the isolation, cloning and transfer of specific genes using the methods of plasmid insertion, splicing and ligation for genetic engineering. In vitro studies will involve development of new cell lines, and studies of the effects of cell nutrition and metabolism of virus replication.

Progress: 87/01 to 87/12. In cooperation with Lim Technology, Richmond, Va., an encapsulation process was tested that did not inactivate baculoviruses. Field studies indicate significant increase in activity of viruses encapsulated with selected dyes compared with viruses encapsulated without dyes or unencapsulated. These results are of interest to scientists and to those companies formulating these microorganisms. Restriction endonuclease mapping of the gypsy moth virus genome was completed and the site of the polyhedrin gene identified. A cell line that permitted partial replication was identified, permitting studies on biochemical factors limiting host range of the nuclear polyhedrosis viruses. A new cell line derived from fat body tissue of the gypsy moth produces significantly more polyhedra when infected with a highly virulent strain of gypsy moth virus. The cell line was adapted to a readily available tissue culture medium (TC-100) to increase its usefulness for production of the virus. Studies with some commercially available serum replacements demonstrated that one of these, CPSR-3, Sigma Chemical, was satisfactory. Cell growth and baculovirus production were equivalent to that in serum supplemented media at 1/3 the cost. Studies demonstrated that there is more variation in virulence of viruses produced in cell cultures than for the same viruses produced in insects. These results are of interest primarily to other scientists.

Publications: 87/01 to 87/12

LYNN, D.E., FELDLAUFER, M.F. and LUSBY, W.R. 1987. Isolation and identification of 20-hydroxyecdysone from a lepidopteran continuous cell line. Arch. Insect Biochem. Physiol. 5:71-79.

MCCLINTOCK, J.T., LYNN, D.E., DOUGHERTY, E.M. and SHIELDS, K. 1987. Embryonic and fat body cell cultures from gypsy moth: characterization and virus susceptibility. In Vitro Cell Dev. Biol. 23:62A. (Abstract).

LYNN, D.E. and OBERLANDER, H. 1986. Obtainment of hormonally sensitive cell lines from imaginal discs of Lepidoptera species. Tech. in the Life Sci., C1, In Vitro Invertebr. Hormones and Genes, C213, 1-12 Els. Sci. Pub., Ireland Ltd.

TOMPKINS, G.J., DOUGHERTY, E.M., ADAMS, J.R., and DIGGS, D. 1987. Changes in virulence of NPV when propagated in alternate noctuid (Lepidoptera:Noctuidae) cell lines and hosts. Accepted by J. Econ. Entomol., Dec. 11, 1987.

DOUGHERTY, E.M., MCCLINTOCK, J.T., and SHIELDS, K. 1987. In vivo infection of L. dispar with a nuclear polyhedrosis virus of Autographa californica (AcNPV). VII Int. Congress of Virology, Aug. 14-19, 1987, Edmonton, Canada p 208 (Abstr).

VAUGHN, J.L., ADAMS, J.R., DOUGHERTY, E.M. and MCCLINTOCK, J.T. 1987. The use of serum replacements in medium for the in vitro production of an insect NPV. VII Int. Congress of Virology, Aug. 14-19, 1987, Edmonton, Canada p 207 (Abstr).

DOUGHERTY, E.M. 1987. Insect viral control agents. Developments in Industrial Microbiology 28:63-75.

65.024 CRIS0048036

DEVELOPMENT OF A STRAIN OF A MULTIPLE EMBEDDED NUCLEAR POLYHEDROSIS VIRUS OF THE GYPSY MOTH

DOUGHERTY E; WEINER R; Agricultural Research Service; University of Maryland, College Park, **MARYLAND** 20742.

Proj. No.: 1275-22240-002-01S
Project Type: COOPERATIVE AGREE.
Agency ID: ARS Period: 28 SEP 82 to 30 SEP 87

Objectives: Characterize existing strains of Lymantria dspar nuclear polyhedrosis viruses by biochemical and biophysical means. Use mutagens to attempt increasing virulence of existing strains and develop novel cell lines for producing and quantitating newly developed viruses.

Approach: Virus strains from selected labs will be examined by restriction endonucle-ase analysis, electron microscopy and polyacrylamide gel electrophoresis. Mutagens will be fed to virus infected insects and virus infected cell cul-tures and progeny virus will be originated from various tissue sources available in Lymantria dispar life stages.

Progress: 87/01 to 87/12. The ARS research effort involving the nuclear polyhedrosis virus of the gypsy moth (LdNPV) has been successfully advanced through the tenure of Dr. McClintock's research associateship. Dr. McClintock's previous experience with the LdNPV system allowed quick progress in accomplishing several goals. Most significant of these is the establishment of a genomic map of LdNPV from restriction endonuclease fragments. This work was accomplished both by cosmid cloning and cross blot hybridization and now allows intelligent manipulation of the LdNPV genome. In addition, it has been shown that no detectible host DNA sequences are transposed in the viral DNA. A genomic library of the host

DNA has been established utilizing cosmid cloning. Several unique proteins identified in LdNPV-insect host interactions can now be isolated, sequenced, and oligomeric probes synthesized. With these, genes can be identified for genetic engineering of the LdNPV. These results are of interest to other scientists who are interested in comparative virus genetics as in the use of viruses for genetic engineering studies.

Publications: 87/01 to 87/12

MCCLINTOCK, J.T. and DOUGHERTY, E.M. 1987. Cosmid cloning and restriction endonuclease mapping of the DNA of the NPV of L. dispar. the gypsy moth. VII Int. Cong. of Virol., Aug. 14-19, 1987, Edmonton, Canada p 207 (Abstr.) MCCLINTOCK, J.T., and DOUGHERTY, E.M. 1987. Superinfection of baculovirus infected gypsy moth cells with the nuclear polyhedrosis viruses of Autographa californica and Lymantria dispar. Virus Research 7:351-364.

65.025 CRIS0130006

BIOSYSTEMATICS OF HELIOTHINE PESTS: MOLECULAR AND MORPHOLOGICAL APPROACHES

MITTER C; Entomology; University of Maryland, College Park, **MARYLAND** 20742.
Proj. No.: MD-8601624 Project Type: CRGO
Agency ID: CRGO Period: 01 SEP 86 to 31 AUG 90

Objectives: PROJECT 8601624. This project will provide well corroborated species definitions and phylogenies for two major pest-containing heliothine noctuid moth groups, Helicoverpa Hardwick and the Heliothis virescens complex. It will also determine what the close relatives of these groups are, by placing them in a phylogeny of the tribe they belong to.

Approach: Three sets of data will be gathered: morphology, of all available life stages; electrophoretic analysis, of 20-25 enzyme markers; mitochondrial DNA restriction maps, based on 20-25 restriction enzymes. The data will be analyzed by numerical phylogenetic techniques.

Progress: 87/01 to 87/12. Accomplishments: Dissections completed for preliminary morphological survey of subfamily Heliothinae, or genus Helicoverpa, and of the tobacco budworm complex. Study of morphometric variation across the range of the tobacco budworm suggests the existence of additional species or subspecies. Surveys begun of verification of allozyme and mitochondrial DNA restriction analyses. Live frozen moths for molecular studies have been accululated for all species groups of Helicoverpa, several species each of Heliothis and Schinia.

Publications: 87/01 to 87/12

NO PUBLICATIONS REPORTED THIS PERIOD.

65.026 CRIS0098217

TOSCANA VIRUS EVOLUTION: IMPLICATIONS FOR USE OF VIRUS VACCINES AND DIAGNOSTIC PROBES

NICHOL S T; School of Veterinary Medicine; University of Nevada, Reno, **NEVADA** 89557.
Proj. No.: NEV00811 Project Type: ANIMAL HEALTH
Agency ID: CSRS Period: 01 JUL 86 to 30 JUN 89

Objectives: To determine the overall rate and content of genetic evaluation of Toscana virus during serial transovarial vertical transmission of the virus in sand flies. To ascertain which virus genes evolve most rapidly and which are highly conserved. To establish the exact sequence of virus genetic changes occurring during these serial infections.

Approach: Analyze evolution of Toscana virus in sand flies by T1 ribonuclease fingerprinting. Compare fingerprints of individual genome segments from each sand fly generation. Sequence RNA virus genomes of Toscana virus and compare with other RNA virus sequences using computer program.

Progress: 86/07 to 89/06. We have completed the sizing and T1 ribonuclease fingerprinting analysis of the RNA genomes of Toscana virus isolates from successive generations of an experimentally virus infected laboratory colony of Phlebotomus perniciosus sandflies. Virus genomes were found to consist of three genome RNA segments. An L segment of 6.5 Kb, and M segment of 4.4 Kb and an S segment of 2.1 Kb. No virus RNA genome changes were detected during transovarial transmission of the virus over 12 sandfly generations (a period of almost 2 years). These results demonstrate that although RNA viruses can exhibit high rates of mutational change under a variety of conditions, some virus RNA genomes are maintained in a stable manner during repeated transovarial virus transmission in the nautral insect host. The apparent stability may be due to the absence of host immune pressure and switching of host environment. This may allow the virus population to remain in a stable equilibrium weighted to the most fit virus. This finding has implications for the development of vaccines and diagnostic probes for insect transmitted RNA viral diseases of agricultural importance.

Publications: 86/07 to 89/06

NO PUBLICATIONS REPORTED THIS PERIOD.

65.027 CRIS0098768

TOSCANA VIRUS EVOLUTION: IMPLICATIONS FOR USE OF VIRUS VACCINES & DIAGNOSTIC PROBES

NICHOL S T; School of Veterinary Medicine; University of Nevada, Reno, **NEVADA** 89557.
Proj. No.: NEV00816 Project Type: HATCH
Agency ID: CSRS Period: 01 JUL 86 to 30 JUN 89

Objectives: To determine the overall rate and content of genetic evaluation of Toscana virus during serial transovarial vertical transmission of the virus in sand flies. To ascertain which virus genes evolve most rapidly and which are highly conserved. To establish

the exact sequence of virus genetic changes occurring during these serial infections.

Approach: Analyze evolution of Toscana virus in sand flies by T1 ribonuclease fingerprinting. Compare fingerprints of individual genome segments from each sand fly generation. Sequence RNA virus genomes of Toscana virus and compare with other RNA virus sequences using computer program.

Progress: 88/01 to 88/12. We have completed the sizing and T1 ribonuclease fingerprinting analysis of the RNA genomes of Toscana virus isolates from successive generations of an experimentally virus infected laboratory colony of Phlebotomus perniciosus sandflies. Virus genomes were found to consist of three genome RNA segments. An L segment of 6.5 Kb, and M segment of 4.4 Kb and an S segment of 2.1 Kb. No virus RNA genome changes were detected during transovarial transmission of the virus over 12 sandfly generations (a period of almost 2 years). These results demonstrate that although RNA viruses can exhibit high rates of mutational change under a variety of conditions, some virus RNA genomes are maintained in a stable manner during repeated transovarial virus transmission in the natural insect host. The apparent stability may be due to the absence of host immune pressure and switching of host environment. This may allow the virus population to remain in a stable equilibrium weighted to the most fit virus. This finding has implications for the development of vaccines and diagnostic probes for insect transmitted RNA viral diseases of agricultural importance.

Publications: 88/01 to 88/12
NO PUBLICATIONS REPORTED THIS PERIOD.

65.028 CRIS0137894
CHARACTERIZATION OF THE MOLECULAR VARIABILITY OF VESICULAR STOMATITIS VIRUS

NICHOL S T; School of Veterinary Medicine; University of Nevada, Reno, **NEVADA** 89557.
Proj. No.: NEV00824 Project Type: HATCH
Agency ID: CSRS Period: 01 JUL 89 to 30 JUN 92

Objectives: To determine the nucleotide sequence of genes coding for the major antigens of eight vesicular stomatitis viruses.

Approach: The experimental approach to be employed includes analysis of the complete nucleotide sequence of the major antigen genes of at least 4 VSV NJ and IND isolates representative of each of the existing subtypes using the primer extension dideoxynucleotide sequencing technique. Computer analysis of the RNA and protein sequences using Microgenie and Ancstr software programs will allow the detailed comparison of these viruses.

65.029 CRIS0137911
CHARACTERIZATION OF THE MOLECULAR VARIABILITY OF VESICULAR STOMATITIS VIRUS

NICHOL S T; School of Veterinary Medicine; University of Nevada, Reno, **NEVADA** 89557.
Proj. No.: NEV00804 Project Type: ANIMAL HEALTH
Agency ID: CSRS Period: 01 JUL 89 to 30 JUN 92

Objectives: To determine the nucleotide sequence of genes coding for the major antigens of eight vesicular stomatitis viruses.

Approach: The experimental approach to be employed includes analysis of the complete nucleotide sequence of the major antigen genes of at least 4 VSV NJ and IND isolates representative of each of the existing subtypes using the primer extension dideoxynucleotide sequencing technique. Computer analysis of the RNA and protein sequences using Microgenie and Ancstr software programs will allow the detailed comparison of these viruses.

65.030 CRIS0097238
GENETIC MARKERS FOR IDENTIFYING GYPSY MOTH POPULATIONS

HARRISON R G; Ecology and Systematics; Cornell University, Ithaca, **NEW YORK** 14853.
Proj. No.: NYC-183311 Project Type: CRGO
Agency ID: CRGO Period: 01 SEP 85 to 31 MAR 89

Objectives: Proj 8502705. Determine if genetic differences exist between gypsy moth populations in geographically separated, but historically similar outbreak sites and if so, determine if differences are sufficient to provide clear genetic markers for recognizing affinities of gypsy moth in adjacent resistant areas.

Approach: To accomplish these objectives, gypsy moth egg masses will be collected from forest sites which have a history of localized flush-crash cycles. The eggs will be hatched and the insect reared to adult. A detailed specific comparison of mitochondrial DNA restriction endonuclease fragment patterns will be made.

Progress: 87/01 to 87/12. Our attention has been directed to the potential use of mitochondrial DNA (mtDNA) as a genetic marker for distinguishing local gypsy moth populations in the United States and throughout the range of the species. To this end, we have completed a detailed restriction site map of a single North American strain and identified site differences between this reference strain and moths from France, China and Japan. To provide higher resolution, we have set up and refined techniques for running arcylamide and high percentage agarose gels with end-labeled restriction endonuclease digests of gypsy moth mtDNA. These techniques will allow us to assay many more restriction fragments in our search for site-specific markers. We have recently finished purifying mtDNA from 18 isofemale lines, representing four sites in New England

(three sites in Vermont and one in Rhode Island). Additional material will become available in the summer. We are just now initiating a complete survey of the 18 mtDNAs in our search for restriction fragment length polymorphisms (RFLPs). The battery of restriction enzymes includes those that recognize four-base sequences (and hence cut more often) and those that recognize A+T-rich sequences (since gypsy moth mtDNA is extremely rich in A+T-rich).

Publications: 87/01 to 87/12
NO PUBLICATIONS REPORTED THIS PERIOD.

65.031 CRIS0130427
PATTERN AND PROCESS IN A NARROW HYBRID ZONE

HARRISON R G; Ecology and Systematics; Cornell University, Ithaca, **NEW YORK** 14853.
Proj. No.: NYC-183305 Project Type: STATE
Agency ID: SAES Period: 01 NOV 86 to 30 SEP 89

Objectives: To understand the genetic structure and dynamics of a hybrid zone between two closely related cricket species, and to elucidate phylogenetic relationships among the eastern North American representatives of the field cricket genus Gryllus.

Approach: Allozymes and mtDNA will be used to assess genetic structure, particularly within the hybrid zone in Connecticut. This will be accompanied by studies of (1) habitat association, (2) post-mating barriers (through lab hybridization studies), and (3) mate choice (female "choice" of males in standard lab cages). Comparison of mtDNA restriction site maps will provide a new data set for evaluating genetic affinities among the entire array of eastern North American Gryllus.

Progress: 87/01 to 87/12. Our efforts have focused on developing a more detailed understanding of the genetic structure of the Gryllus firmus/Gryllus pennsylvanicus hybrid zone and on using mtDNA restriction site maps to construct a phylogeny of North American field crickets. The hybrid zone studies have involved (1) analysis of mtDNA restriction fragment length polymorphisms (RFLPs) to document relationships among populations of G. firmus and G. pennsylvanicus at several sites along the extensive hybrid zone and (2) initiating studies of RFLPs of nuclear gene markers within and adjacent to the hybrid zone in Connecticut. The new mtDNA data confirm our previous assertion that two distinct lineages are in contact in Connecticut. In the southeast, however, the hybridizing taxa differ by only one restriction site. Patterns of variation suggest extensive introgression of G. pennsylvanicus mtDNA into G. firmus populations. We have constructed a representative genomic library in a lambda phage replacement vector and have begun to screen the library for sequences that can be used as markers in the hybrid zone. The mtDNA phylogeny of eastern North American crickets (based on comparison of restriction site maps) indicates that there are four distinct clades, each characterized by a different life cycle. Within each clade (life cycle), very recent speciation has led to the current diversity of species. We are in the process of completing the data set for the eastern crickets and are beginning work on a set of western species.

Publications: 87/01 to 87/12
NO PUBLICATIONS REPORTED THIS PERIOD.

65.032 CRIS0131529
POPULATION GENETICS OF INTRODUCED INSECT PESTS

HARRISON R G; Ecology and Systematics; Cornell University, Ithaca, **NEW YORK** 14853.
Proj. No.: NYC-183404 Project Type: HATCH
Agency ID: CSRS Period: 01 APR 87 to 30 SEP 90

Objectives: The primary objective is to use molecular markers to document patterns of genetic variation within and among populations of two important introduced pests, the European corn borer and the gypsy moth.

Approach: The initial phase of the project involves extending ongoing studies of mtDNA variation in both species by applying recently introduced high-resolution techniques for screening restriction-fragment-length polymorphisms. mtDNA is a particularly good marker for tracing the spread of introduced species. The new techniques combine feasibility with the ability to screen variation at a very large number of restriction sites. I will also initiate studies comparing restriction fragment patterns and restriction site maps for selected nuclear gene sequences.

Progress: 87/04 to 87/12. We have devoted most of the initial phase of this project to establishing protocols for (1) constructing and screening genomic libraries in lambda replacement vectors and (2) end-labeling DNA fragments necessary for running high-resolution acrylamide gels. We have purified mtDNA from selected gypsy moth isofemale lines that will be used in our search for genetic variants that can serve as markers.

Publications: 87/04 to 87/12
NO PUBLICATIONS REPORTED THIS PERIOD.

65.033 CRIS0136809
SYSTEMATICS OF THE PRODOXIDAE AND THE PHYLOGENY OF HOST ASSOCIATIONS AND MUTUALISM

HARRISON R G; Ecology and Systematics; Cornell University, Ithaca, **NEW YORK** 14853.
Proj. No.: NYC-183321 Project Type: STATE
Agency ID: SAES Period: 01 JAN 89 to 30 JUN 92

Objectives: The aim of this project is to gain a detailed understanding of the evolutionary relationships among genera within the family Prodoxidae (Lepidoptera) and among species within the prodoxid genus Greya. These data serve as a framework for understanding the phylogeny of host associations and mutualism between these moths and their host plants. The prodoxids include the yucca moths and related genera, and include taxa often cited as classic

examples of coevolution between insects and plants.

Approach: Direct sequencing of selected regions of prodoxid mitochondrial DNA and restriction site mapping of the entire mitochondrial genome will provide the data from which we will infer phylogenies.

65.034 CRIS0138394
GENETIC ANALYSIS OF AN INSECT HYBRID ZONE

HARRISON R G; Ecology and Systematics; Cornell University, Ithaca, **NEW YORK** 14853.
Proj. No.: NYC-183324 Project Type: STATE
Agency ID: SAES Period: 15 OCT 89 to 30 SEP 93

Objectives: The intent is to provide a detailed genetic analysis of a hybrid zone between two closely related cricket species. I wish to characterize opportunities for and constraints on genetic exchange at several sites along the hybrid zone. In addition I will continue to investigate the nature of post-mating barriers to gene exchange and the basis for mate selection.

Approach: The approach will be to use restriction fragment length polymorphisms (RFLPs) of single-copy nuclear DNA, in conjunction with analysis of mtDNA and morphology, to examine patterns of variation at individual loci and patterns of linkage disequilibrium between pairs of loci. Lab mate choice experiments and test crosses will provide data on barriers to gene exchange.

65.035 CRIS0096512
ORGANIZATION AND SIGNIFICANCE OF NATURALLY OCCURRING GENETIC VARIATION IN DROSOPHILA MELANOGASTER

AQUADRO, C F; Genetics Development; Cornell University, Ithaca, **NEW YORK** 14853.
Proj. No.: NYC-186419 Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 85 to 30 SEP 89

Objectives: What is the level, nature, organization and significance of naturally occurring DNA sequence variation in drisiphila melanogaster? Does a correlation exist between transcriptional activity and the level of DNA sequence polymorphism? Is there evidence of insertional hotspots for transposable elements? How is the variation at different genes along the sequence organized (randomly or is there evidence for selectively favored combinations). What is the molecular basis for naturally occurring quantitative variation in expression of genes in these regions.

Approach: Nucleotide variation will be assayed over the 315,000 base pair rosy to Ace region and the 400,000 base pair Bithorax Complex region of D. melanogaster by constructing and comparing restriction endonuclease maps from lines of flies obtained from several natural populations. Additional detailed restriction mapping and DNA sequencing will focus on a 10,000 base pair region containing the gene for xanthine dehydrogenase and two adjacent genes. Levels of gene expression at rosy and Ace, as well as other genes, will be assessed by enzyme activity and messenger RNA levels. The molecular sources of variation in gene expression will be investigated by genetic crossed and in vitro mutagenesis and transformation.

Progress: 88/01 to 88/12. We have nearly completed our analysis of the levels, pattern and organization of naturally occurring restriction map variation across the 315kb rosy/Ace region of D. melanogaster. Discovery of strong linkage disequilibrium across the 315kb region and extending some 6.6 map units to the Bithorax complex led us to extend our analysis to additional regions on the third, second and X chromosomes of population samples of D. melanogaster and of the homologous regions in D. simulans and D. pseudoobscura. DNA variation is are distributed nonrandomly across genes and chromsomes due to differences in constraint, mutation rate and natural selection. DNA variation is 4-6 times higher in D. simulans and D. pseudoobscura, compared to D. melanogaster. This difference is in striking contrast to levels of protein variation. Transposable elements contribute significantly less to naturally occurring sequence variation in both D. simulans and D. pseudoobscura compared to D. melanogaster. High levels of linkage disequilibrium were observed between DNA variants as much as 10.8 map units apart in D. melanogaster. Analysis of data for scattered restrictions site variants on the X, 2nd and 3rd chromosomes of D. melanogaster and D. simulans, and for the 3rd and 4th chromosomes of D. pseudoobscura shows a positive correlation between extensive linkage disequilibrium and the presence of polymorphic chromosome inversions.

Publications: 88/01 to 88/12

SCHAEFFER, S.W., AQUADRO, C.F. and LANGLEY, C.H. 1988. Restriction map variation in the Notch locus of D. melanogaster. Mol. Biol. Evol. 5:30-40.

WESTNEAT, D.F., NOON, W.A., REEVE, H.K. and AQUADRO, C.F. 1988. Improved hybridization conditions for DNA fingerprints probed with M13. Nucl. Acids Res. 16:4161.

LANGLEY, C.H., SHRIMPTON, A.E, YAMAZAKI, T., MIYASHITA, N., MATSUO, Y. and AQUADRO, C.F. 1988. Naturally occurring variation in the restriction map of the Amy region of D. melanogaster. Genetics 119:619-629.

AQUDRO, C.F., LADO, K.M. and NOON, W.A. 1988. The rosy region of D. melanogaster and D. simulans. I. Contrasting levels of naturally occurring DNA restriction map variation and divergence. Genetics 119:875-888.

PARRISH, C.R., AQUADRO, C.F. and CARMICHAEL, L.E. 1988. Canine host range & a spec. epitope map along w/variant seqs. in the capsid protein gene of canine parvovirus & related feline, mink & raccoon parvoviruses.Virol.166:293-307.

AQUADRO, C.F., WEAVER, A., SCHAEFFER, S.W. and ANDERSON, W.W. 1988. Molecular evolution of inversions in D. pseudoobscura the amylase gene region. Proc. Natl. Acad. Sci. USA, in press.

65.036 CRIS0088324
CHARACTERIZATION OF THE DROSOPHILA ZESTE LOCUS GENE PRODUCT

GOLDBERG, M L; Genetics Development; Cornell University, Ithaca, **NEW YORK** 14853.
Proj. No.: NYC-186416 Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 86 to 30 SEP 89

Objectives: To achieve a molecular understanding of the process of transvection in Drosophila melanogaster, and to define theasis of the interaction between the zeste locus and unlinked complex loci in the Drosophila genome.

Approach: Polypeptides defined by the nucleotide sequence of the zeste locus will be employed for two purposes. First, antibodies directed against zeste/beta-galactosidase fusion proteins will be generated, and used to define the intracellular location of the zeste locus product and the distribution of this protein in the tissues of the developing animal. Second, zeste protein synthesized in bacterial cells or in vitro translational systems will be used to examine hypothesis concerning the molecular role played by this protein. In particular, we will explore the possibility that such synthetic protein willbind to DNA sequences defined by genetic studies to be required for zeste interactions.

Progress: 88/01 to 88/12. Within the past year, we have learned a considerable amount of information concerning the function and structure of the zeste gene product. We have determined that the zeste protein binds specifically to DNA sequences at the white locus required for genetic interaction with zeste. The region of the zeste protein involved in this interaction was localized to a position between amino acids 47 and 138 relative to the N terminus, based upon activity of beta-galactosidase fusion proteins containing segments of the zeste protein. We have shown in cotransfection experiments using Drosophila tissue culture cells that the zeste protein can activate in trans the transcription of several genes, including the Ultrabithorax(Ubx) transcription unit of the biothorax complex. Interestingly, zeste appears to decrease the expression of the Antennapedia gene, suggesting that this protein can activate the transcription of some genes while repressing that of others. We have recently found that the C-terminal region of zeste is required for the activation of Ubx. This region contains a structure related to leucine zippers, and may be involved in protein-protein interactions underlying the phenomenon of transvection.

Publications: 88/01 to 88/12

MANSUKHANI, A., GUNARATNE, P.H., SHERWOOD, P.W., SNEATH, B.J. and GOLDBERG, M.L. 1988. Nucleotide sequence & structural analysis of the zeste locus of D. melanogaster. Mol. Gen. Genet. 211:121-128.
MANSUKHANI, A., CRICKMORE, A., SHERWOOD, P.W. and GOLDBERG, M.L. 1988. DNA-binding properties of the D. melanogaster zeste gene product. Mol. Cell. Biol. 8:615-623.

65.037 CRIS0094088
GENETIC ANALYSIS OF EARLY EMBRYOGENESIS IN C. ELEGANS

KEMPHUES K J; Genetics Development; Cornell University, Ithaca, **NEW YORK** 14853.
Proj. No.: NYC-186417 Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 88 to 30 SEP 91

Objectives: Continue genetic and phenotypic analyses of the four previously identified loci required for asymmetric cytoplasmic partitioning during early development of C. elegans. Isolate multiple alleles at each locus. Construct double mutants to learn about interactions among the genes. Correlate early and late defects to learn more about the relationship between specific early embryonic events and determination of intestine and germ line. Examine the cellular basis of the maternal-effect sterility exhibited by mutants at three of the four loci. Identify and characterize additional genes required for cytoplasmic localization or determination of the intestine lineage in particular. Isolate DNA encoding genes required for asymmetric partitioning in C. elegans.

Approach: Analyses will include large scale mutant hunts to isolate multiple mutations in each of the four known genes, mutant hunts to identify additional partitioning genes, examination of mutant phenotypes by light microscopy, time-lapse video tape recording, immunocytochemistry, and cell lineage analysis. DNA corresponding to these genes will be isolated by chromosome "walking" and transposon "tagging". In addition, mutant hunts to identify maternal-effect lethal mutants that result in failure to execute the developmental program leading to the formation of intestine will be performed.

Progress: 88/01 to 88/12. We have continued our genetic analysis of maternally acting genes required for cytoplasmic localization in C. elegans and have initiated molecular studies of these genes. We have identified eight new alleles of the five par genes and nearly completed our phenotypic characterization. We have extended our phenotypic analysis to the very early stages in development for four of the five par genes and have shown that mutants in all four genes are defective in pseudocleavage (a transient partial construction of the media cortex) and microfilament distribution during the first cell cycle. These results are consistent with the view that the par genes are required for some important aspect of cytoskeletal structure during the first cell cycle. We have begun molecular cloning of the genes par-3 and par-4. We have genetically mapped RFLPs that identify start sites for chromosome walking as a prelude to isolating par-3 DNA. We have also identified a putative transposon insertion into the gene

par-4 which, when verified, will serve as a start site for chromosome walking. In addition, we have succeeded in cloning the gene zyg-11, another gene required for proper cytoplasmic organization in early C. elegans embryos.

Publications: 88/01 to 88/12

KEMPHUES, K.J. 1988. Genetic analysis of embryogenesis in Caenorhabditis elegans. IN Developmental Genetics of Higher Organisms (G.M. Malacinski, ed.), pp. 193-219, McMillan, New York.

KEMPHUES, K.J., PRIESS, J.R., MORTON, D.G. and CHENG, N. 1988. Identification of genes required for cytoplasmic localization in early C. elegans embryos. Cell 52:311-320.

KEMPHUES, K.J., KUSCH, M. and WOLF, N. 1988. Maternal-effect lethal mutations on linkage group II in C. elegans. Genetics 120:977-986.

KEMPHUES, K.J. 1988. The early development of Caenorhabditis elegnas. IN Frontiers in Molecular Biology: Early Development (David M. Glover, ed.), IRL Press, London, in press.

CHENG, N., MORTON, D.G. and KEMPHUES, K.J. 1988. Analysis of a gene required for cytoplasmic localization in E. elegans (Poster). XVIth International Congress of Genetics.

65.038 CRIS0130039

REPLICATION AND GENE EXPRESSION OF TRICHOPLUSIA NI GRANULOSIS VIRUS IN CELL CULTURE

GRANADOS R R; Biological Control; Tower Road, Ithaca, **NEW YORK** 14853.
Proj. No.: NYR-8600350 Project Type: CRGO
Agency ID: CRGO Period: 01 SEP 86 to 31 AUG 88

Objectives: Project 8600350. To provide information on the gene organization and expression of Trichoplusia ni granulosis virus (GV) under in vitro conditions.

Approach: We will improve and utilize existing, susceptible T. ni embryonic cell line for replication of GV in vitro. These cultures will permit temporal studies of viral DNA, mRNA, and protein synthesis. A physical map for the GV genome will be constructed by cosmid cloning and analysis of the GV-DNA. Viral transcripts will be mapped by hybridization selection and their protein products identified by in vitro translation.

Progress: 86/09 to 87/12. A physical map of Trichoplusia ni granulosis virus (TnGV) DNA, which has a genome size of 180 Kbp, was obtained with HindIII restriction enzyme mapping and vectors of a cosmid pH C79 and a plasmid pBluescript. During the course of this study, we found that some HindIII clones in pBluescript vectors could faintly hybridize to several other HindIII fragments as well as to themselves under a reduced stringency condition, suggesting TnGV DNA may have interspersed homologous regions similar to some NPVs. Approximately 100 new T. ni cell lines, which were established from T. ni eggs during the last 2 years were screened for their susceptibility to TnGV. No cell lines showed significant ability to support TnGV replication.

Publications: 86/09 to 87/12

GRANADOS, R.R. and HASHIMOTO, Y (in press). Infectivity of Baculoviruses to Cultured Cells. In: Invertebrate Cell System Applications, Vol. II (Ed. J. Mitsuhashi). CRC Press, Boca Raton, FL.

65.039 CRIS0099733

PERSISTENT LDMNPV INFECTION OF GYPSY MOTHS IN NATURE

WOOD H A; Boyce Thompson Institute; Tower Road, Ithaca, **NEW YORK** 14853.
Proj. No.: NYR-8601486 Project Type: CRGO
Agency ID: CRGO Period: 01 SEP 86 to 28 FEB 89

Objectives: PROJECT 8601486. To provide information concerning nature and impact of persistent Lymantria dispar (gypsy moths).

Approach: We will investigate the conditions required for transovarial transmission of LdMNPV and the establishment of persistent LdMNPV infections in animals and tissue culture cells. Once established, we will investigate factors which induce productive virus replication in persistently infected tissues.

Progress: 87/09 to 89/02. Using cosmid and plasmid cloning DNA hybridization procedures, we constructed a physical map of a cloned isolate of LdMNPV with 6 restriction enzymes. The LdMNPV genome contains 166.6 kilobase pairs. We identified and sequenced the coding and flanking regions of the polyhedrin gene. The polyhedrin gene contained the highly conserved 14 base sequence (promoter sequence) which is common to all NPV described to date. The gene has a coding region of 735 bases and adeduced amino acid sequence with a high degree of homology to other NPV polyhedrons. The LdMNPV polyhedrin transcripts are 3.5 kb in length. The LdMNPV genome was also found to contain 5 regions of highly repeated (hr) sequences. Using dot-blot DNA:DNA hybridization procedures, it has been shown that approximately 2-3% of the field collected Lymantria dispar egg masses are positive for the LdMNPV. According transovarial transmission of LdMNPV does occur, but can only be detected at a low rate. Using laboratory reared gypsy moth larvae obtained from the USFS Hamden labs, we stressed larvae with several chemical and nonhost viruses. Approximately 10% of the larvae were judged to be latently infected based on the induction of productive replication of LdMNPV in larvae stressed with either congo red or the Autographa californica nuclear polyhedrosis virus.

Publications: 87/09 to 89/02

WOOD, H.A., BURAND, J.P., HUGHES, P.H., FLORE, P.H. and GETTIG, R.R. 1986. Transovarial transmission of Lymantria dispar nuclear polyhedrosis virus. In Fundamental and Applied Aspects of Invertebrate Pathology.

SMITH, I.R.L.,VAN BEEK, N.A.M., PODGWAITE, J.D. and WOOD, H.A. 1988. Physical map and polyhedrin gene sequence of Lymantria dispar nuclear polyhedrosis virus. Gene 71:97-106.
SMITH, I.R.L., VAN BEEK, N.A.M., PODGWAITE, J.D. and WOOD, H.A. In preparation. Size and location of LdMNPV polyhedrin gene transcripts.

65.040* CRIS0085099
REGULATION OF GENE EXPRESSION DURING EUCARYOTIC DEVELOPMENT

BEWLEY G C; Genetics; North Carolina State University, Raleigh, **NORTH CAROLINA** 27695.
Proj. No.: NC03762 Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 81 to 30 SEP 87

Objectives: Determine any structural differences betewwn the isozymes of glycerol-3-phosphate dehydrogenase in Drosophila. Determine the manner in which the expression of GPDH isozymes are controlled during development. Isolate and chracterize genetic variants affecting the developmental program of GPDH expression.

Approach: Each purified isozyme is to be subjected to a structural analysis including tryptic digestion, generation of peptide maps, purification of peptides from each map with a subsequent compositional and sequence analysis. Genetic variants of the developmental program of GPDH will be characterized by mapping studies, protein turnover studies and an analysis of the steady-state protein.

Progress: 78/10 to 87/09. Glycerol-phosphate dehydrogenase in Drosophila melanogaster consists of a family of three isozymes which exhibit a unique temporal and tissue-specific pattern of expression. Even though these isozymes are encoded by the same structural gene, they differ in the amino acid sequence at the C-terminal end. We have isolated both genomic and cDNA clones in order to examine the structure of the 3'-end of this gene and its transcriptional products. The results of this analysis demonstrate that this gene represents a complex transcriptional unit whereby the isozymic forms of GPDH are encoded by three classes of transcripts each generated by developmentally regulated 3'-end formations and alternate splicing pathways of the pre-mRNA. We have just completed a genetic screen which has resulted in the isolation of six null mutants for the enzyme catalase. These mutants are currently being characterized. The mutants will provide us with the raw material to analyze the role of catalase in oxygen free radical metabolism and the role of oxygen radicals in promoting genetic damage.

Publications: 78/10 to 87/09
SHAFFER, J. B., SUTTON, R. B. and G. C. BEWLEY. 1987. Isolation of a cDNA clone for murine catalase and analysis of an acatalasemic mutant. J. Biol. Chem. 262:12908-12911.

65.041 CRIS0135583
P ELEMENT-INDUCED QUANTITATIVE VARIATION IN DROSOPHILA MELANOGASTER

MACKAY T F C; Genetics; North Carolina State University, Raleigh, **NORTH CAROLINA** 27695.
Proj. No.: NC06077 Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 88 to 30 SEP 91

Objectives: To use P element mutagenesis in Drosophila melanogaster to induce quantative genetic variation; to identify and clone quantitative trait loci (QTL); to correlate molecular and phenotypic variation at QTL.

Approach: A large number of X and third chromosome lines will be constructed which differ only in the number and location of stable P transposable element insertions, in a highly inbred genetic background originally free of P elements. These lines will be analyzed at the phenotypic level for P-induced quantitative genetic variatioin for bristle traits, longevity, fertility and total fitness, and at the molecular level for the number and cytological locations of the P insertions. QTL will thus be identified and cloned and the analysis extended to variation at these loci in natural and highly selected populations.

65.042 CRIS0061162
CYTOGENETIC STUDIES IN THE NEMATODE FAMILY HETERODERIDAE

TRIANTAPHYLLOU A C; Genetics; North Carolina State University, Raleigh, **NORTH CAROLINA** 27695.
Proj. No.: NC03334 Project Type: HATCH
Agency ID: CSRS Period: 01 JUL 71 to 30 SEP 86

Objectives: Elucidate phyletic relationships among species of root-knot and cyst nematodes through analysis and evaluation of the chromosomal complement, chromosome pairing patterns, mode of reproduction and DNA content per nucleus. Evaluate ultrastructural behavior of pachytene chromosomes in an attempt to elucidate evolution of parthenogenesis, polyploidy and aneuploidy.

Approach: Gametogenesis and reproduction studies, including analyses of chromosomal complements and measurements of DNA will be carried out in several hundred populations of root-knot and cyst nematodes, as well as other members of the families Heteroderidae and Meloidogynidae. Naturally occurring diploid, aneuploid and polyploid forms and other forms produced in the laboratory following hybridization will be compared cytologically employing light and transmission electron microscopy. Analyses of karyotypes by reconstruction of synaptonemal complexes will be carried out in selected populations.

Progress: 71/07 to 86/09. Cytogenetic studies of 800 populations representing 16 species indicated that evolution of root-knot nematodes has been influenced by extensive modifications of their karyotype involving the

establishment of polyploidy and aneuploidy in association with parthenogenetic reproduction. Diploid, amphimictic species (e.g. Meloidogyne megatyla - with n = 18 chromosomes) are least successful as plant parasites. Aneuploid, meiotically parthenogenetic species (M. hapla, M. chitwoodi - with n = 14 - 17) are very successful parasites adapted to temperate and cold climates. Polyploid, apomictic species (M. incognita, M. arenaria, M. javanica - with 2n = 30 - 56) are extremely successful parasites adapted to temperate and subtropical climates. Enzyme phenotypes from 300 populations originating from 65 countries provided reliable characters for identification of Meloidogyne species. A phylogenetic tree based on variation in 27 enzymes suggested that apomictic species share a common phyletic lineage, and that meiotically parthenogenetic and amphimictic species are not closely related to each other, or to the apomictic species. Cot curves derived from renaturation kinetics of sheared, denatured DNA indicated that the genome of Meloidogyne species is composed of about 20% repetitive and 80% unique sequences. DNA-DNA hybridization and thermal elution tests, using radioactively-labeled 32p-DNAs suggested that M. incognita, M. javanica and M. arenaria are closely related, with 80 to 100% homology to two probes.

Publications: 71/07 to 86/09

ESBENSHADE, P. R. and TRIANTAPHYLLOU, A. C. 1986. Partial characterization of esterases in Meloidogyne (Nematoda). Comp. Biochem. Physiol. 83B:31-36.

GOLDSTEIN, P. and TRIANTAPHYLLOU, A. C. 1986. The synaptonemal complex of Meloidogyne nataliei and its relationship to that of other Meloidogyne species. Chromosama (Berl). 93:261-266.

TRIANTAPHYLLOU, A. C. Cytogenetic status of Meloidogyne (Hypsoperine) spartinae in relation to other Meloidogyne species. J. Nematol. (In press).

ESBENSHADE, P. R. and TRIANTAPHYLLOU, A. C. Enzymatic relationships and evolution in the Genus Meloidogyne (Nematoda: Tylenchida). J. Nematol. (In press).

TRIANTAPHYLLOU, A. C. Cytogenetic approaches in nematode taxonomy and identification. Proceedings of the 1st International Nematology Workshop, held in Karachi, Pakistan (April 6-8, 1986).

TRIANTAPHYLLOU, A. C. Biochemical approaches in Nematode taxonomy and identification. Proceedings of the 1st International Nematology Workshop, held in Karachi, Pakistan (April 6-8, 1986).

65.043 CRIS0132682
GENETICS OF PARASITISM OF THE SOYBEAN CYST NEMATODE

TRIANTAPHYLLOU A C; ESBENSHADE P R; Genetics; North Carolina State University, Raleigh, **NORTH CAROLINA** 27695.
Proj. No.: NC09347 Project Type: CRGO
Agency ID: CRGO Period: 01 AUG 87 to 31 JAN 90

Objectives: Proj. 8700338. Construct a genetic linkage map of the H. glycines genome based on isozyme markers. Identify specific genetic factors controlling parasitism in various inbred lines of the nematode and specify their positions on the enzymatic linkage map.

Approach: Selected populations of H. glycines differing in their parasitic abilities and isozyme markers will be subjected to controlled, single-pair matings in order to study mode of inheritance of various enzymes & determine recombination frequencies between enzymic loci. This information will allow determination of linkage groups and development of a genetic map of the H. glycines genome. To this map, specific genes for parasitism will be added following an analysis of the segregation patterns of progeny in controlled crosses of well-defined inbred lines with specific genes for parasitism linked to specific enzyme phenotypes.

Progress: 88/01 to 88/12. The genetic basis of esterase polymorphism in Heterodera glycines was investigated through controlled matings of three selected-inbred lines, each fixed for a different esterase phenotype. F1 progeny were homogeneous, exhibiting both parental esterase phenotypes (codominant heterozygotes) but no hybrid bands. About 1,500 progeny segregated in a 1:2:1 ratio suggesting that the three esterase phenotypes correspond to three codominant alleles of a single esterase locus. Using esterase phenotypes as genetic markers we demonstrated the occurrence of multiple matings by both males and females of H. glycines. Crosses between isolates that differ in ability to reproduce on resistant soybeans (PI-88788 and Pickett) suggested a nonrecessive type of inheritance of the ability of the nematode to overcome resistance. Three-factor crosses between isolates differing in two enzymic markers and in ability to reproduce on the resistant soybean PI 88788 indicated that some enzymes may be linked to genes for parasitism. These tests are not yet completed.

Publications: 88/01 to 88/12

ESBENSHADE, P.R. and TRIANTAPHYLLOU, A.C. 1988. Genetic analysis of esterase polymorphism in the soybean cyst nematode, Heterodera glycines. Journal of Nematology 20:486-492.

65.044 CRIS0134336
NEURONAL CONTROL MECHANISMS IN NEMATODES

OPPERMAN C H; Plant Pathology; North Carolina State University, Raleigh, **NORTH CAROLINA** 27695.
Proj. No.: NC06060 Project Type: HATCH
Agency ID: CSRS Period: 01 APR 88 to 30 SEP 92

Objectives: Isolate and characterize molecular forms of acetylcholinesterase from Meloidogyne spp., Heterodera spp., and selected other plant-parasitic nematodes; identify and isolate acetylcholine receptor subtypes from Caenorhabditis elegans and M. incognita; identify molecular components of the GABAergic system in nematodes.

Approach: Molecular forms of acetylcholinesterase will be isolated by a combination of density gradient centrifugation and ion exchange chromatography. Both physical and biochemical properties will be evaluated by standard methods. Response to inhibitors will be investigated. Receptor subtypes will be identified through competitive binding assays for pharmacological characterization. Receptors will be isolated by affinity chromatography. A combination of genetic manipulation and pharmacological perturbation will be used to identify GABAergic system components; we will use mutations/resistance to infer drug function, and drugs to identify specific gene alterations.

Progress: 88/04 to 88/12. Drug screening experiments have been initiated to examine the role of biogenic amine neurotransmitters in control of nematode behavior. Several classes of drugs have thus far been active on C. elegans, and of these we have chosen to work in two systems. The adrenergic system may have a significant role in initiation of the moulting process. The octopaminergic system function is not known at this time, although it could be involved in both sensory and neuromuscular controls. A catecholamine receptor has been isolated from C. elegans by using affinity chromatography. Characterization and antisera production are proceeding. Chemical mutagenesis has yielded nineteen drug-resistant strains for the octopaminergic system, although no resistance has been found thus far to the adrenergic drugs. Genetic mapping has been initiated.

Publications: 88/04 to 88/12

OPPERMAN, C.H. 1988. Neurological control of molting in Caenorhabditis elegans. J. Nematology 20:654-655. (abstract).

65.045 CRIS0131463

GENETIC TRANSFORMATION OF A SOIL NEMATODE BY MICROINJECTION OF CLONED DNA

MILLER D M; Zoology; North Carolina State University, Raleigh, **NORTH CAROLINA** 27695.
Proj. No.: NC05644 Project Type: STATE
Agency ID: SAES Period: 01 OCT 86 to 30 APR 88

Objectives: The objective of this proposal is to develop a reliable method for inserting genes into the chromosomes of a soil nematode. We intend to employ this technique to identify DNA sequences that regulate the expression of nematode genes. The information to be gained from this project is fundamentally important to our understanding of the genetic specification of nematode development.

Approach: Cloned genes will be microinjected into oocyte nuclei in the gonad of a mature nematode. The host embryo is genetically disabled in such a way that it cannot reproduce unless the injected DNA is incorporated into its genome. Thus, when fertile offspring arise from the injected parent then the exogenous DNA has been successfully inserted into the genome. In this way, nematode genes that have been chemically modified in the laboratory can be assayed for the effects of these changes in vivo.

Progress: 87/05 to 88/04. We have employed a simple but powerful selection scheme to isolate spontaneous movement defective mutants from a C. elegans strain (TR679) exhibiting high transposition frequencies. Synchronized populations of TR679 larvae were placed in the middle of a nutrient agar plate with a circumferential lawn of bacteria. Animals that could not crawl to food within two hours were removed from the plate for outcrossing and genetic mapping. More than 20 independent mutant strains were isolated. Mutations in the unc-4 gene alter synaptic input to one class of motor neurons in the ventral nerve cord; eight of twelve VA motor neurons assume the connectivity pattern normally reserved for the VB class of motor neurons. As a result, unc-4 mutants exhibit a characteristic "uncoordinated" phenotype -- the inability to crawl backwards. We intend to clone this homeotic gene by a chromosomal walking technique from closely linked transposon insertion sites that we have recently identified. Once we have deduced the DNA sequence of the unc-4 gene it should be possible to formulate a molecular model to explain its role in the fundamental phenomenon of neural specificity.

Publications: 87/05 to 88/04

NO PUBLICATIONS REPORTED THIS PERIOD.

65.046 CRIS0131464

MOLECULAR GENETIC ANALYSIS OF A MYOSIN HEAVY CHAIN GENE EXPRESSION

MILLER D M; Zoology; North Carolina State University, Raleigh, **NORTH CAROLINA** 27695.
Proj. No.: NC05645 Project Type: STATE
Agency ID: SAES Period: 01 OCT 86 to 30 JUN 88

Objectives: The objective of this project is to dissect the genetic mechanisms regulating the expression of myosin heavy chain (MHC), a major protein component of muscle. DNA sequences adjacent to a nematode MHC will be analyzed to search for putative regulatory or promoter sites. Mutations that alter these regions and which affect MHC gene expression will be sequenced in order to identify the specific regulatory domains.

Approach: We have designed a genetic scheme that selects for mutants expressing elevated levels of MHC in the nematode. With this approach, we hope to isolate mutations in DNA sequences that regulate MHC expression. The specific lesions associated with each mutant will be determined by DNA sequencing methods.

Progress: 86/10 to 88/06. In the nematode, C. elegans, the body wall muscles contain paramyosin and two different types of myosin heavy chain, MHC A and MHC B. In mutants that are null for MHC B or which express defective paramyosin, muscle structure is disrupted and movement is impaired. Second site mutations in the sup-3 locus partially reverse these defects and are correlated with a 2-3 fold increase in

the accumulation of the MHC A isoform. Sup-3 mutations occur at a high frequency (1/10,000) that is comparable to the average mutation rate per gene after ethylmethane sulfonate (EMS) mutagenesis. We have shown that sup-3 mutants correspond to amplification of the structural gene for the MHC A protein, myo-3. We employed genomic Southern hybridization with MHC gene specific probes in order to measure the copy number of the myo-3 gene relative to that of the MHC B gene, unc-54. We have identified the putative amplification junctions for two of these sup-3 alleles using a set of cosmid clones which encompass the myo-3 region. Although it has been suggested that gene amplification plays an important role in evolution, there are few known cases of gene amplification in the germ line cells of multicellular organisms. The results shown here provide a clear example of a heritable gene amplification event that occurs at high frequency and may thus represent the initial step in the evolution of new function and in the formation of multigene families.

Publications: 86/10 to 88/06

MILLER, D. M. and MARUYAMA, I. 1986. The sup-3 locus is closely linked etc. In Molecular Biology of Muscle Development. C. Emerson, et al (Eds) (Alan R. Liss, New York). pp 629-638.

MILLER, D.M., STOCKDALE, F. and KARN, J. 1986. Immunological identification of the genes encoding the four myosin heavy chains of Caenorhabditis elegans. Proc. Natl. Acad. Sci. 83:2305-2309.

65.047 0014893

BIOCHEMICAL AND PHYSIOLOGICAL BASES FOR THE DEVELOPMENT OF BIORATIONAL INSECTICIDES

FEYEREISEN R; Entomology; Oregon State University, Corvallis, **OREGON** 97331.
Proj. No.: ORE00090 Project Type: HATCH
Agency ID: CSRS Period: 01 JUL 89 to 30 JUN 94

Objectives: To study the biochemistry of insect juvenile hormone biosynthesis in insects and its physiological and neurohormonal control. To study the molecular biology of insect cytochrome P450 in insects, with particular emphasis on the role of P450 enzymes in insecticide resistance, the induction of P450 enzymes by plant chemicals, and the physiological functions of P450 enzymes in insects. To apply this knowledge in the quest for biorational insect control methods based on the disruption of essential, insect-specific biochemical and physiological processes.

Approach: These objectives will be pursued in studies of cytochrome P450 monooxygenases at the biochemical and molecular level in the house fly, Musca domestica; in studies of juvenile hormone synthesis in the cockroach, Diploptera punctata.

Progress: 88/01 to 88/12. Lepidoptera (including many major agricultural pests) synthesize the homoisoptrenoid juvenile hormone II and JH I, whereas all other insects synthesize only JH III, a normal isoprenoid. We have shown that the failure to generate propionate or propionyl-CoA is the key determinant of the homolog specificity of juvenile hormone synthesis in non-lepidopteran insects. This observation may lead to the development of juvenile hormone synthesis inhibitors specific for Lepidoptera. We have cloned and sequenced the first insect cytochrome P450 gene, following the successful screening of a cDNA expression library from insecticide-resistant house flies. This cytochrome P450 is likely to play an important role in the resistance of insects to several classes of insecticides. Its deduced amino acid sequence spans 509 amino acids (molecular weight 58,738) and is distantly related (27% overall identity) to vertebrate liver P450 III proteins which are involved in the metabolism of macrolide antibiotics.

Publications: 88/01 to 88/12

FEYEREISEN, R. and FARNSWORTH, D. E. (1988). Forced synthesis of trace amounts of juvenile hormone II from propionate by corpora allata of a juvenile hormone III-producing insect. Experientia 44:47-49.

BARTON, A. E. , WING, K. D. , LEE, D. P. , SIAWECKI, R. A., and FEYEREISEN, R. Arylpyridyl-thiosemicarbazones: a new class of anti-juvenile hormones active against lepidoptera. Experientia (in press).

BELAI, I., MATOLCSY, G., FARNSWORTH, D. E., and FEYEREISEN, R. Inhibition of insect cytochrome P450 by some metyrapone analogues and compounds containing a cyclopropylamine moiety. Pesticide Science (in press).

FEYEREISEN, R., KOENER, J. F., FARNSWORTH, D. E., and NEBERT, D. W. Isolation and sequence of cDNA encoding a cytochrome P450 from an insecticide-resistant strain of the house fly Musca domestica. Proc. Natl. Acad. Sci. USA (in press).

NEBERT, D. W., NELSON, D. R., and FEYEREISEN, R. The evolution of cytochrome P450 genes. Xenobiotica (in press).

65.048 CRIS0135720

CLONING AND ANALYSIS OF GENES FOR INSECTICIDE RESISTANCE

BROWN T M; Entomology; Clemson University, Clemson, **SOUTH CAROLINA** 29634.
Proj. No.: SC01287 Project Type: HATCH
Agency ID: CSRS Period: 01 SEP 88 to 30 JUN 93

Objectives: Determine the molecular nature of genetic mutations which confer insecticide resistance in the tobacco budworm, Heliotis virescens (F.) and other pests of field crops in order to understand the genesis of resistance. Prepare alternative chemical strategies in anticipation of phyrethroid resistance by characterizing the selectivity of resistant mutants toward conventional and novel insecticides. Improve knowledge of the population genetics of resistance by developing surveillance methods which detect specific mutant alleles in the field.

Approach: Gene cloning and sequencing techniques (Maniatis et al. 1982) will be employed to identify changes in mutant alleles for resistance to pyrethroid and organophosphorus insecticides. Resistance mechanisms for analysis include the -aminobutyric acid receptor, acetylcholinesterase, acetylcholine receptor, microsomal monooxygenase, carboxylester hydrolase and glutathione transferase. Eggs will be exposed to treated paper, larvae will be treated topically and adults will be exposed in treated glass vials. Enzyme activity and inhibition assays, enzyme-linked immunosorbent assays for protein molecules by antibodies which to which a tracer enzyme has been bound, and DNA assays will be developed to detect resistant insects.

Progress: 88/09 to 88/12. Two gene libraries of Heliothis virescens have been prepared. Other libraries will be prepared for other strains with characterized resistance. Current efforts are directed at cloning and sequencing the gene for the enzyme acetylcholinesterase which is insensitive in one strain of Heliothis virescens in this laboratory. Several heterologous cDNA probes have been obtained for other genes which are known resistance mechanisms. Preliminary results with heterologous probes suggest that it may be necessary to obtain synthetic oligonucleotide probes of highly conserved regions of those genes from other species in order to obtain the analogous genes from Heliothis virescens. Contingency plans include the use of expression systems coupled to assays for activity or antibody recognition in order to obtain the genes of interest from cDNA libraries.

Publications: 88/09 to 88/12
NO PUBLICATIONS REPORTED THIS PERIOD.

65.049 CRIS0132766
BASIC AND APPLIED GENETIC LINKAGE MAPPING IN HELIOTHIS VIRESCENS

HECKEL D G; BROWN T M; Entomology; Clemson University, Clemson, **SOUTH CAROLINA** 29634.
Proj. No.: SC-8702153 Project Type: CRGO
Agency ID: CRGO Period: 01 AUG 87 to 31 JUL 90

Objectives: PROJ. 8702153. Verify the genetic nature of 20 putative enzyme polymorphisms and determine their linkage relationships. Screen 40 additional enzymes for polymorphism. Synthesize one marker strain susceptible to pyrethroids. Genetically map pyrethroid resistance.

Approach: Pedigree analysis of families started from pairs of field-collected individuals will be followed by specific crosses to determine linkage using rare allozymes determined by starch gel electrophoresis. Frozen samples from pedigree collection will be analyzed for additional polymorphisms. Resistant strains will be analyzed for allozymes and a susceptible strain will be produced from pedigree families which has alternative allozymes. Hybrids of the resistant to susceptible cross will be backcrossed and progeny analyzed for genotype and resistance to determine linkage.

Progress: 88/01 to 88/12. Twenty putative enzyme polymorphisms listed in the grant proposal have been examined. One was shown to be sex-linked. Six were shown to be autosomal and mutually unlinked. Of the remaining 13 putative enzyme polymorphisms, variation was documented at three of them but was shown to be non-genetic in nature. The remaining 10 enzymes were not polymorphic in our lab colony of Heliothis virescens and thus linkage relationships could not be determined. Fourteen of 40 additional enzymes listed in the proposal have been screened for polymorphisms. Two of these were shown to be sex-linked, and five are autosomal. Enzyme activity was present for an additional four, but these were not polymorphic in our laboratory colony. The remaining three enzymes showed no activity under our assay conditions. The PTJ strain of Heliothis virescens was susceptible to pyrethroids and was polymorphic for nine enzyme polymorphisms. Although not fully homozygous for rare electrophoretic alleles, it was a useful strain carrying enzyme markers for nearly one-third of the genome. It was used to test for possible linkage of enzyme loci to pyrethroid resistance in crosses with a resistant strain. The pyrethroid resistance locus was not linked to any of the nine marker loci in the PTJ strain.

Publications: 88/01 to 88/12
NO PUBLICATIONS REPORTED THIS PERIOD.

65.050 CRIS0142544
GENETIC LINKAGE MAPS FOR INHERITANCE OF INSECTICIDE RESISTANCE

RIDGWAY R L; HECKEL D G; BROWN T M;
Entomology; Clemson University, Clemson, **SOUTH CAROLINA** 29634.
Proj. No.: 1275-24000-014-02S
Project Type: COOPERATIVE AGREE.
Agency ID: ARS Period: 30 SEP 87 to 31 MAY 90

Objectives: To develop genetic linkage maps for insecticide resistance in insects such as Heliothis spp., using enzyme polymorphisms as markers and thus to establish the basis for determining patterns of inheritance for this resistance.

Approach: Enzyme polymorphisms will be used to test for linkage of genes in Heliothisvirescens. If enzyme markers are exhausted, then restriction fragment length polymorphism will be used to complete the map. Pedigree analysis andplanned crosses will be used. Techniques employed will include starch and polyacrylamide eletrophoresis techniques and a computer program which has been developed for pedigree records.

Progress: 88/01 to 88/12. Three polymorphic enzyme loci have been mapped to the Z-chromosome by virtue of being sex-linked. Two loci are separated by about 28 centimorgans, and these two have not yet been previously mapped in any Lepidopteran to our knowledge. When these results are combined with our other

linkage mapping studies (funded by a USDA Competitive Grant), we now have approximately 25% of the 31 chromosomes marked with polymorphic enzyme loci. The PTJ strain, derived from a collection of larvae from tobacco in May 1987, has been maintained continuously by single-pair matings since that time. The current (tenth) generation is polymorphic for the three sex-linked enzyme loci discussed above, as well as seven additional autosomal loci. This is the only multiply-marked strain of Heliothis virescens to our knowledge. We are currently maintaining this strain for our future use and that of other workers. One cDNA probe from Drosophila melanogaster containing a putative single-copy gene has been screened for hybridization with DNA from H. virescens. No hybridization was detected, indicating that there has probably been much evolutionary divergence between these two species for this particular gene. Three hundred and sixteen clones from a plasmid library of H. virescens genomic DNA were subjected to colony hybridization with total Heliothis DNA. Sixty-two percent of these were eliminated as highly repeated sequences unsuitable for restriction fragment length polymorphism (RFLP) studies.

Publications: 88/01 to 88/12
NO PUBLICATIONS REPORTED THIS PERIOD.

65.051 CRIS0099038
PLANT NEMATODE MITOCHONDRIAL DNA

WOLSTENHOLME D R; Biology; University of Utah, Salt Lake City, **UTAH** 84112.
Proj. No.: UTAR-8600094 Project Type: CRGO
Agency ID: CRGO Period: 01 JUN 86 to 31 MAY 89

Objectives: Proj. 8600094. To devise a diagnostic test for identification of different species and races of root knot nematodes (Meloidogyne species).

Approach: We will determine the entire nucleotide sequence of the mitochondrial (mt) DNA molecule of M. javonica, and from this sequence generate a complete restriction enzyme cleavage map by computer analysis. Restriction maps of other Meloidogyne species (M. arenaria, M. incognita and M. hapla) and races will be made using restriction enzyme cleavage and gel electrophoresis. We will then work out specific conditions to use radioactively-labeled, cloned fragments of M. javonica mtDNA in a capillary blot hybridization procedure to identify the different species and races of Meloidogyne.

Progress: 88/01 to 88/12. We have sequenced 18.2 kb of the estimated total 20 kb of the mitochondrial DNA (mtDNA) molecule of the root knot nematode Meloidogyne javanica. Within a 13 kb continuous segment of this sequence we have identified genes for the small and large ribosomal RNAs, 12 proteins and 12 transfer RNAs (tRNAs). All of these genes would be transcribed in the same direction. The arrangement of these genes is greatly different from the arrangements of homologous genes found in other metazoan mtDNAs, including those of two nematodes Caenorhabiditis elegans and Ascaris suum. Nine of the tRNA genes of M. javanica mtDNA have secondary structures similar to those of most C. elegans and A. suum mitochondrial tRNAs: that is, they lack a TpsiC arm and variable loop. Between three gene-containing sequences (totalling 1840 ntp) of M. incognita mtDNA that we have obtained, and the corresponding sequences of M. javanica mtDNA, there are only two nucleotide substitutions (0.1%). Within a sequence of approximately 7 kb of M. javanica mtDNA tha appears to lack genes, are two sets of directly repeated sequences; eleven copies of a 63 ntp sequence, and about 30 copies of a 102 ntp sequence. The 102 ntp repeat sequence also occurs in mtDNAs of M. incognita, M. hapla and M. arenaria. By hybridizing a radioactively-labeled, cloned DNA that includes two copies of the M.

Publications: 88/01 to 88/12
NO PUBLICATIONS REPORTED THIS PERIOD.

65.052 CRIS0097253
MOLECULAR ANALYSIS OF ECLOSION HORMONE IN THE TOBACCO HORNWORM, MANDUCA SEXTA

REDDIFORD L M; TRUMAN J W; Zoology; University of Washington, Seattle, **WASHINGTON** 98195.
Proj. No.: WNR-8500521 Project Type: CRGO
Agency ID: CRGO Period: 15 AUG 85 to 31 AUG 88

Objectives: Proj 8500521. To obtain and sequence the gene for eclosion hormone so as to deduce its amino acid sequence which will then be confirmed by synthesis. To study the developmental and hormonal regulation of eclosion hormone gene expression.

Approach: The eclosion hormone (EH) antibody will be used to pull the EH cDNA clone from a lambda gt11 expression vector library of cDNA from developing Manduca adult brains. This cDNA clone will then be used to obtain the EH genomic clones from the Manduca genomic library. These will then be sequenced, and the corresponding amino acid sequence deduced. This peptide will be synthesized and its biological activity tested. The cDNA and/or genomic clones will be used for hybridization analysis with nervous system RNAs from different stages of development and in response to ecdysteroid levels. The presence of EH-like sequences in other insects will be analyzed by Southern hybridization.

Progress: 88/01 to 88/12. We isolated the ecolsion hormone (EH) gene from a genomic library of the tobacco hornworm, Manduca sexta using a 72 nucleotide-long oligonucleotide probe which was designed based on the known sequence of the peptide. We confirmed the identity of the EH gene by nucleic acid sequence analysis. The portion of the gene which we have sequenced corresponds to amino acid 5 to 62, the C-terminus of the peptide. Following the C-terminus is a termination codon indicating that any additional peptides that might be present on the polyprotein precursor must be derived upstream from the N-terminius of EH. We have not yet located the putative upstream exon and we are currently using a

number of approaches to obtain this sequence. We have constructed a cDNA library from developing adult brain mRNA and we are currently screening this library for the EH cDNA clone using the genomic clone as a probe. Hybridization of the EH gene to a Southern blot of digested Manduca DNA shows that the gene is single copy in the Manduca genome. We have determined by Northern blot analysis that the gene is expressed in the brain throughout adult development as a 940 nt mRNA. This mRNA is absent in tissues of non-neural origin. We recently showed that this mRNA is present in larval brains, but not in other locations in the nervous system. We are currently determining the cellular location of the EH mRNA by in situ hybridization.

Publications: 88/01 to 88/12

REBERS, J., HORODYSKI, F., HICE, R. and RIDDIFORD, L.M. 1987. "Genomic clones of developmentally regulated larval cuticle genes from the tobacco hornworm, Manduca sexta" in Molecular Entomology (J. Law, ed.), UCLA Symp. Mol. Cell Biol.

HORODYSKI, F.M., TRUMAN, J.W. and RIDDIFORD, L.M. 1988. Isolation of the eclosion hormone gene from the tobacco hornworm, Manduca sexta. Abstract for XVIII International Congress of Entomology. Vancouver, B.C.

TRUMAN, J.W., MORTON, D., HORODYSKI, F.M. and RIDDIFORD, L.M. 1988. The molecular biology of ecolsion hormone: From synthesis to action. Abstract for Workshop on Molecular Biology and Molecular Genetics of Lepidoptera, Kolymbari, Crete.

HORODYSKI, F.M., TRUMAN, J.W. and RIDDIFORD, L.M. 1989. Isolation of the eclosion hormone gene from the tobacco hornworm, Manduca sexta. Abstract for ASCB/ASBMB Meeting, San Francisco., in preparation.

65.053* CRIS0136166
GENETIC AND MOLECULAR APPROACHES TO HONEY BEE IMPROVEMENT

MILNE C P JR; Entomology; Washington State University, Pullman, **WASHINGTON** 99164.

Proj. No.: WNP00818 Project Type: STATE
Agency ID: SAES Period: 01 SEP 88 to 31 AUG 91

Objectives: Develop methods for direct gene transfer into the honey bee. Develop methods to map cloned genes in the honey bee. Conduct short-term research relevant to the Washington beekeeping industry.

Approach: Clone and characterize honey bee transposable elements. Clone and evaluate honey bee genes as candidate reporter genes for transformation. Clone and characterize promoters for tissue, stage and sex specific expression. Evaluate potential beneficial genes for transfer to the honey bee. Perfect the in situ hybridization technique of cloned genes to metaphase chromosomes. Identify specific genomic clones that hybridize to each honey bee chromosome. Develop a bank of chromosome specific genomic clones that saturate the genome. Perfect the Southern mapping technique of cloned genes on honey bee chromosomes. Meet with relevant parties to ascertain acute needs of the Washington beekeeping industry that can be addressed by published research findings or short-term research projects.

65.054 CRIS0135927
STUDIES OF THE INSECT PATHOGENIC BACTERIUM XENORHABDUS LUMINESCENS

NEALSON K H; FRACKMAN S; Board of Regents; University of Wisconsin, Milwaukee, **WISCONSIN** 53201.

Proj. No.: WISR-8800776 Project Type: CRGO
Agency ID: CRGO Period: 01 JUL 88 to 30 JUN 90

Objectives: PROJ. 8800776. The objectives of the proposed research are to elucidate several aspects of the physiology of Xenorhabdus luminescens, a bacterium which is pathogenic to many insects. We will focus on understanding the mechanism whereby the primary (virulent) form of Xenorhabdus is converted to the secondary (avirulent) form. This will include both the definition of the physiological conditions that determine primary/secondary conversion, and the genetic or molecular alterations that occur upon conversion.

Approach: We will take advantage of the fact that the primary forms are bioluminescent, while the secondary forms are not, and use this marker for physiological and molecular genetic and physiological studies. Physiological approaches will involve media manipulations, and nutrient limited growth studies; genetic studies will involve the use of standard techniques of cloning, transformation, sequencing and mapping, using the already-cloned genes for bioluminescence from Xenorhabdus.

65.055 CRIS0132894
MOLECULAR HOST-PARASITE INTERACTIONS IN THE TOBACCO HORNWORM

BECKAGE N E; Entomology; University of Wisconsin, Madison, **WISCONSIN** 53706.

Proj. No.: WIS03171 Project Type: CRGO
Agency ID: CRGO Period: 01 SEP 87 to 31 AUG 90

Objectives: Proj. 8700241. Cotesia congregata is a braconid wasp parasite of the tobacco hornworm, Manduca sexta. The female parasite injects a double-stranded DNA virus into the host larva, along with eggs, during parasitization of the host. A novel 33 kd polypeptide appears in the hemolymph within 2 to 4 hours post-parasitization and is induced in unparasitized larvae by the injection of virus isolated from the ovarian calyces of Cotesia. Thus this protein plausibly represents a viral gene product or a host gene product induced by the virus in the absence of parasites.

Approach: The goals of this project are to examine the temporal pattern of viral gene expression and characterize viral gene products; and to determine the amino acid sequence of the 33 kd polypeptide with the intent of assessing whether the gene which encodes for it lies within the host, parasite, or viral genome(s). Its regulation can then be studied under conditions of normal development of the wasps versus parasite encapsulation. Standard protein sequencing techniques will be used to obtain a partial sequence of the protein, then the likely DNA sequence encoding for the protein can be deduced. Viral sequences will be cloned and used for screening host tissues for viral replication.

Progress: 88/01 to 88/12. The braconid wasp Cotesia congregata injects a double-stranded DNA virus (polydnavirus) into host tobacco hornworm larvae, along with parasite eggs which develop in the hemocoel. In the past year we have purified and characterized this polydnavirus. Its genome is comprised of 17-24 double-stranded DNA circles ranging in size from 4 to >20 kb. Viral structural polypeptides were characterized using polyacrylamide gel electrophoresis. Northern blotting experiments are underway to clarify the temporal pattern of viral gene expression in the host, along with characterizing the role of viral gene products in modulating the host-parasite interaction. Detailed hemolymph polypeptide studies were carried out to characterize parasitism-specific polypeptides in naturally parasitized larvae, for comparison to proteins produced in unparasitized larvae injected with purified Cotesia polydnavirus and controls injected with inactivated virus. Both 1-D and 2-D gel analyses were employed, along with C4 reverse phase HPLC, to characterize hemolymph proteins. Our goal is to determine if the virus alone may be exploited as a biological pesticide to cause death of pest insects, circumventing the need for utilization of the parasites themselves. Development of parasite teratocytes, which are cells arising from the dissociation of the serosal cell layer encasing the embryo, was characterized using SEM, TEM, and flourescence activated cell sorter analyses.

Publications: 88/01 to 88/12

BECKAGE, N.E., METCALF, J.S., NIELSON, B.D. and NESBIT, D.J. 1988. Disruptive effects of azadirachtin on development of Cotesia congregata in host tobacco hornworm larvae. Arch. Insect Biochem. Physiol. 9:47-65.

BECKAGE, N.E., NESBIT, D.J., NIELSEN, B.D., SPENSE, K.D. and BARMAN, M.A.E. 1988. Parasitic alteration of hemolymph polypeptides in Manduca sexta: a two-dimensional electrophoretic analysis and comparison.

THOMPSON, S.N., LEE, R.W.R. and BECKAGE, N.E. 1988. Metabolism of parasitized Manduca sexta examined by nuclear magnetic resonance. Arch. Insect Biochem. Physiol. In press.

THOMPSON, S.N., LEE, R.W.K. and BECKAGE, N.E. 1988. Characterization of the _3_1P spectrum Manduca sexta and effects of antimetabolites. Insect Biochem. In press.

BECKAGE, N.E., METCALF, J.S., NESBIT, D.J., SCHLEIFER, K.W. and ZETLAN, S.R. 1988. Parasitic inhibition of insect host hemolymph monophenoloxidase. Submitted to PNAS.

BECKAGE, N.E. 1988. Parasitic effects on host development. Chapter for UCLA Symposium Series book entitled New Directions in Biological Control. In preparation (to be submitted January, 1989.)

SCHLEIFER, K.S. 1988. Characterization of the genome and viral polypeptides of Cotesia congregata polydnavirus, and evidence for transcription of viral genes.

66.001* CRIS0136380
GENETICS OF LYMANTRIA DISPAR AND ITS BACULOVIRUS

KNUDSON D L; Colorado State University, Fort Collins, **COLORADO** 80523.
Proj. No.: COLR-8802297 Project Type: CRGO
Agency ID: CRGO Period: 01 SEP 88 to 31 AUG 90

Objectives: PROJ. 8802297. The overall objective of this proposal is to examine this interaction at a genetic level using the gypsy moth, Lymantria dispar, and its viral system, L. dispar multiple-embedded nuclear polyhedrosis virus (LdMNPV); with the goal of using this knowledge to improve the potency and efficacy of a baculovirus pesticide.

Approach: The experimental approaches will include the construction of a genomic library of LdMNPV which will be used to generate a physical map of LdMNPV and its genomic variants. Regions on the genome where variation occurs will be identified. A genomic library of the host will be constructed and used in the identification of host cell inserts in the viral genome. The cellular DNA inserts will be characterized to determine whether they are expressed in uninfected cells. The cellular inserts will be sequenced, and their copy number in L. dispar will be determined. The viral insertional regions will be examined to determine the effect that the insertion of host cell DNA has on viral transcription, and these results will be compared with transcription in virulent wild-type virus infections in vitro.

Progress: 88/01 to 88/12. The E. coli toxin, STIa, causing infant and traveler's diarrhea in humans and collibacilosis in cattle, sheep and pigs, is an 18 amino acid peptide which is too small to be recognized by the immune system. At present, there is no effective vaccine or prophylactic treatment against STIa-mediated diarrheal disease. We are using two approaches to provide protection against the effects of this toxin. First, a synthetic gene has been constructed which links repeating units (encoding 17 of the 18 amino acids) of the STIa gene to produce a "multimeric" gene capable of expressing a large, antigenic protein which should have multiple epitopes in common with the native toxin. We have cloned "multimeric genes" which encode from two to ten linked-copies of the monomeric "core" sequence. The recombinant proteins will be tested for immune response, toxicity and neutralizing ability. Second, we have constructed a synthetic analog of the toxin gene. Putatively important amino acids have been deliberately modified through changes at the DNA level to produce a non-toxic STIa-analog protein. The "non-functional" gene-encoded protein will be compared to activity of a synthetic "wildtype" gene we have constructed. Using insertional sequences, we will attempt to insert this non-functional gene in place of the active toxin-producing gene to construct a biological control organism which would be capable of colonizing the gut but would be incapable of producing an active STIa-toxin.

Publications: 88/01 to 88/12
No publications reported this period.

66.002* CRIS0134012
GENE SYNTHESIS AND EXPRESSION OF PROTEIN PRODUCTS FOR NATURAL AND MUTANT HEAT-STABLE ENTEROTOXIN

TRUMBLE W R; Bacteriology & Biochemistry; University of Idaho, Moscow, **IDAHO** 83843.
Proj. No.: IDA00911 Project Type: HATCH
Agency ID: CSRS Period: 01 JAN 88 to 31 DEC 92

Objectives: Constructing a synthetic gene and expressing active heat-stable enterotoxin (ST-1) and inactive mutant analog peptides. The synthetic gene(s) will be used to attempt to make the enterotoxin immunoreactive for vaccine development and to allow homologous recombination experiments to produce an infective E. coli incapable of producing active enterotoxin for use as a veterinary treatment to prevent enterotoxigenic EV coli diarrheal disease.

Approach: The published amino acid sequence of the ST-1 peptide has been back translated into nucleotide sequence. Several important design features have been incorporated into the gene design. Synthetic oligonucleotides will be constructed containing mutations for specific amino acids and after combining the oligonucleotides to produce double strand ST-1 genes, DNA probes will be used to isolate genes encoding native and mutant ST-1 peptides.

66.003* CRIS0048495
ARTHROPOD VIRUSES: CHARACTERIZATION, GENETICS AND REPLICATION IN VIVO AND IN VITRO

VAUGHN J L; TOMPKINS G J; ADAMS J R; Insect Pathology Lab Plant Protection Inst; Beltsville Agr Res Center, Beltsville, **MARYLAND** 20705.
Proj. No.: 1275-22240-002-00D
Project Type: INHOUSE
Agency ID: ARS Period: 29 JUN 83 to 29 JUN 88

Objectives: To characterize viruses from insect pests and evaluate their potential for use in pest management. To determine biochemical processes of virus invasion, replication and virulence and the genetic factors controlling these processes. To develop the necessary in vitro systems for the isolation, replication and production of insect pathogenic viruses.

Approach: Characterization will be done by a combination of light and electron microscope studies and by biochemical analysis of the structural components of the virus. Genetic relatedness to other viral isolates from the same or similar insect species will be determined by restriction endonuclease analysis of the viral genome and by nucleic hybridization tests. Genetic manipulation will be either by classical methods of viral recombination or the isolation, cloning and transfer of specific genes using the methods of plasmid insertion, splicing and ligation for genetic engineering. In vitro studies will

involve development of new cell lines, and studies of the effects of cell nutrition and metabolism of virus replication.

Progress: 87/01 to 87/12. In cooperation with Lim Technology, Richmond, Va., an encapsulation process was tested that did not inactivate baculoviruses. Field studies indicate significant increase in activity of viruses encapsulated with selected dyes compared with viruses encapsulated without dyes or unencapsulated. These results are of interest to scientists and to those companies formulating these microorganisms. Restriction endonuclease mapping of the gypsy moth virus genome was completed and the site of the polyhedrin gene identified. A cell line that permitted partial replication was identified, permitting studies on biochemical factors limiting host range of the nuclear polyhedrosis viruses. A new cell line derived from fat body tissue of the gypsy moth produces significantly more polyhedra when infected with a highly virulent strain of gypsy moth virus. The cell line was adapted to a readily available tissue culture medium (TC-100) to increase its usefulness for production of the virus. Studies with some commercially available serum replacements demonstrated that one of these, CPSR-3, Sigma Chemical, was satisfactory. Cell growth and baculovirus production were equivalent to that in serum supplemented media at 1/3 the cost. Studies demonstrated that there is more variation in virulence of viruses produced in cell cultures than for the same viruses produced in insects. These results are of interest primarily to other scientists.

Publications: 87/01 to 87/12

LYNN, D.E., FELDLAUFER, M.F. and LUSBY, W.R. 1987. Isolation and identification of 20-hydroxyecdysone from a lepidopteran continuous cell line. Arch. Insect Biochem. Physiol. 5:71-79.

MCCLINTOCK, J.T., LYNN, D.E., DOUGHERTY, E.M. and SHIELDS, K. 1987. Embryonic and fat body cell cultures from gypsy moth: characterization and virus susceptibility. In Vitro Cell Dev. Biol. 23:62A. (Abstract).

LYNN, D.E. and OBERLANDER, H. 1986. Obtainment of hormonally sensitive cell lines from imaginal discs of Lepidoptera species. Tech. in the Life Sci., C1, In Vitro Invertebr. Hormones and Genes, C213, 1-12 Els. Sci. Pub., Ireland Ltd.

TOMPKINS, G.J., DOUGHERTY, E.M., ADAMS, J.R., and DIGGS, D. 1987. Changes in virulence of NPV when propagated in alternate noctuid (Lepidoptera:Noctuidae) cell lines and hosts. Accepted by J. Econ. Entomol., Dec. 11, 1987.

DOUGHERTY, E.M., MCCLINTOCK, J.T., and SHIELDS, K. 1987. In vivo infection of L. dispar with a nuclear polyhedrosis virus of Autographa californica (AcNPV). VII Int. Congress of Virology, Aug. 14-19, 1987, Edmonton, Canada p 208 (Abstr).

VAUGHN, J.L., ADAMS, J.R., DOUGHERTY, E.M. and MCCLINTOCK, J.T. 1987. The use of serum replacements in medium for the in vitro production of an insect NPV. VII Int. Congress of Virology, Aug. 14-19, 1987, Edmonton, Canada p 207 (Abstr).

DOUGHERTY, E.M. 1987. Insect viral control agents. Developments in Industrial Microbiology 28:63-75.

66.004 CRIS0099853

DEVELOPMENT OF AN EFFICIENT PROTEIN SECRETION SYSTEM IN STREPTOCOCCUS FAECALIS

DUNNY G M; WILSON D B; Microbiology; Cornell University, Ithaca, **NEW YORK** 14853.
Proj. No.: NYCV-433-323 Project Type: STATE
Agency ID: CSVM Period: 01 JUL 86 to 30 JUN 89

Objectives: The goal of this project is to develop a Streptococcus faecalis host-plasmid combination that causes the high level production and excretion of cloned genes.

Approach: The proposed research will focus on a protein called C130 that mediates a surface exclusion function in S. faecalis (expression of surface exclusion prevents donor-donor mating in a plasmid transfer system). In S. faecalis, induction of the synthesis of C130 results in its accumulation in the culture fluid. We propose to use recombinant DNA techniques to identify the portion of the C130 protein involved in secretion. We intend to maximize expression of this gene S. faecalis and to develop a system of fusing various foreign genes to the engineered derivative of C1300 in order to cause efficient production and secretion of important proteins.

Progress: 88/01 to 88/12. Our efforts focused on cloning and sequencing the plasmid-encoded gene encoding a protein, Tra 130 of enterococcus faecalis (Streptococcus faecalis) that would be used in the secretion system. We mapped the location of the structural gene, and have subconed the gene into sequencing vectors. Sequencing of the gne is currently in progress.

Publications: 88/01 to 88/12

CHRISTIE, P., KAO, S.-M., ADSIT, J., and DUNNY, G. 1988. Cloning and expression of genes encoding pheromone-inducible antigens of Enterococcus (Streptococcus) faecalis. J.Bacteriol. 170:5161-5168.

66.005* CRIS0130039

REPLICATION AND GENE EXPRESSION OF TRICHOPLUSIA NI GRANULOSIS VIRUS IN CELL CULTURE

GRANADOS R R; Biological Control; Tower Road, Ithaca, **NEW YORK** 14853.
Proj. No.: NYR-8600350 Project Type: CRGO
Agency ID: CRGO Period: 01 SEP 86 to 31 AUG 88

Objectives: Project 8600350. To provide information on the gene organization and expression of Trichoplusia ni granulosis virus (GV) under in vitro conditions.

Approach: We will improve and utilize existing, susceptible T. ni embryonic cell line for replication of GV in vitro. These cultures will permit temporal studies of viral DNA, mRNA, and protein synthesis. A physical map for the GV

genome will be constructed by cosmid cloning and analysis of the GV-DNA. Viral transcripts will be mapped by hybridization selection and their protein products identified by in vitro translation.

Progress: 86/09 to 87/12. A physical map of Trichoplusia ni granulosis virus (TnGV) DNA, which has a genome size of 180 Kbp, was obtained with HindIII restriction enzyme mapping and vectors of a cosmid pH C79 and a plasmid pBluescript. During the course of this study, we found that some HindIII clones in pBluescript vectors could faintly hybridize to several other HindIII fragments as well as to themselves under a reduced stringency condition, suggesting TnGV DNA may have interspersed homologous regions similar to some NPVs. Approximately 100 new T. ni cell lines, which were established from T. ni eggs during the last 2 years were screened for their susceptibility to TnGV. No cell lines showed significant ability to support TnGV replication.

Publications: 86/09 to 87/12

GRANADOS, R.R. and HASHIMOTO, Y (in press). Infectivity of Baculoviruses to Cultured Cells. In: Invertebrate Cell System Applications, Vol. II (Ed. J. Mitsuhashi). CRC Press, Boca Raton, FL.

66.006* CRIS0131463

GENETIC TRANSFORMATION OF A SOIL NEMATODE BY MICROINJECTION OF CLONED DNA

MILLER D M; Zoology; North Carolina State University, Raleigh, **NORTH CAROLINA** 27695.
Proj. No.: NC05644 Project Type: STATE
Agency ID: SAES Period: 01 OCT 86 to 30 APR 88

Objectives: The objective of this proposal is to develop a reliable method for inserting genes into the chromosomes of a soil nematode. We intend to employ this technique to identify DNA sequences that regulate the expression of nematode genes. The information to be gained from this project is fundamentally important to our understanding of the genetic specification of nematode development.

Approach: Cloned genes will be microinjected into oocyte nuclei in the gonad of a mature nematode. The host embryo is genetically disabled in such a way that it cannot reproduce unless the injected DNA is incorporated into its genome. Thus, when fertile offspring arise from the injected parent then the exogenous DNA has been successfully inserted into the genome. In this way, nematode genes that have been chemically modified in the laboratory can be assayed for the effects of these changes in vivo.

Progress: 87/05 to 88/04. We have employed a simple but powerful selection scheme to isolate spontaneous movement defective mutants from a C. elegans strain (TR679) exhibiting high transposition frequencies. Synchronized populations of TR679 larvae were placed in the middle of a nutrient agar plate with a circumferential lawn of bacteria. Animals that could not crawl to food within two hours were removed from the plate for outcrossing and genetic mapping. More than 20 independent mutant strains were isolated. Mutations in the unc-4 gene alter synaptic input to one class of motor neurons in the ventral nerve cord; eight of twelve VA motor neurons assume the connectivity pattern normally reserved for the VB class of motor neurons. As a result, unc-4 mutants exhibit a characteristic "uncoordinated" phenotype -- the inability to crawl backwards. We intend to clone this homeotic gene by a chromosomal walking technique from closely linked transposon insertion sites that we have recently identified. Once we have deduced the DNA sequence of the unc-4 gene it should be possible to formulate a molecular model to explain its role in the fundamental phenomenon of neural specificity.

Publications: 87/05 to 88/04

NO PUBLICATIONS REPORTED THIS PERIOD.

66.007* CRIS0131464

MOLECULAR GENETIC ANALYSIS OF A MYOSIN HEAVY CHAIN GENE EXPRESSION

MILLER D M; Zoology; North Carolina State University, Raleigh, **NORTH CAROLINA** 27695.
Proj. No.: NC05645 Project Type: STATE
Agency ID: SAES Period: 01 OCT 86 to 30 JUN 88

Objectives: The objective of this project is to dissect the genetic mechanisms regulating the expression of myosin heavy chain (MHC), a major protein component of muscle. DNA sequences adjacent to a nematode MHC will be analyzed to search for putative regulatory or promoter sites. Mutations that alter these regions and which affect MHC gene expression will be sequenced in order to identify the specific regulatory domains.

Approach: We have designed a genetic scheme that selects for mutants expressing elevated levels of MHC in the nematode. With this approach, we hope to isolate mutations in DNA sequences that regulate MHC expression. The specific lesions associated with each mutant will be determined by DNA sequencing methods.

Progress: 86/10 to 88/06. In the nematode, C. elegans, the body wall muscles contain paramyosin and two different types of myosin heavy chain, MHC A and MHC B. In mutants that are null for MHC B or which express defective paramyosin, muscle structure is disrupted and movement is impaired. Second site mutations in the sup-3 locus partially reverse these defects and are correlated with a 2-3 fold increase in the accumulation of the MHC A isoform. Sup-3 mutations occur at a high frequency (1/10,000) that is comparable to the average mutation rate per gene after ethylmethane sulfonate (EMS) mutagenesis. We have shown that sup-3 mutants correspond to amplification of the structural gene for the MHC A protein, myo-3. We employed genomic Southern hybridization with MHC gene specific probes in order to measure the copy number of the myo-3 gene relative to that of the MHC B gene, unc-54. We have identified the

putative amplification junctions for two of these sup-3 alleles using a set of cosmid clones which encompass the myo-3 region. Although it has been suggested that gene amplification plays an important role in evolution, there are few known cases of gene amplification in the germ line cells of multicellular organisms. The results shown here provide a clear example of a heritable gene amplification event that occurs at high frequency and may thus represent the initial step in the evolution of new function and in the formation of multigene families.

Publications: 86/10 to 88/06

MILLER, D. M. and MARUYAMA, I. 1986. The sup-3 locus is closely linked etc. In Molecular Biology of Muscle Development. C. Emerson, et al (Eds) (Alan R. Liss, New York). pp 629-638.

MILLER, D.M., STOCKDALE, F. and KARN, J. 1986. Immunological identification of the genes encoding the four myosin heavy chains of Caenorhabditis elegans. Proc. Natl. Acad. Sci. 83:2305-2309.

66.008* CRIS0135927

STUDIES OF THE INSECT PATHOGENIC BACTERIUM XENORHABDUS LUMINESCENS

NEALSON K H; FRACKMAN S; Board of Regents; University of Wisconsin, Milwaukee, **WISCONSIN** 53201.

Proj. No.: WISR-8800776 Project Type: CRGO
Agency ID: CRGO Period: 01 JUL 88 to 30 JUN 90

Objectives: PROJ. 8800776. The objectives of the proposed research are to elucidate several aspects of the physiology of Xenorhabdus luminescens, a bacterium which is pathogenic to many insects. We will focus on understanding the mechanism whereby the primary (virulent) form of Xenorhabdus is converted to the secondary (avirulent) form. This will include both the definition of the physiological conditions that determine primary/secondary conversion, and the genetic or molecular alterations that occur upon conversion.

Approach: We will take advantage of the fact that the primary forms are bioluminescent, while the secondary forms are not, and use this marker for physiological and molecular genetic and physiological studies. Physiological approaches will involve media manipulations, and nutrient limited growth studies; genetic studies will involve the use of standard techniques of cloning, transformation, sequencing and mapping, using the already-cloned genes for bioluminescence from Xenorhabdus.

66.009* CRIS0132894

MOLECULAR HOST-PARASITE INTERACTIONS IN THE TOBACCO HORNWORM

BECKAGE N E; Entomology; University of Wisconsin, Madison, **WISCONSIN** 53706.

Proj. No.: WIS03171 Project Type: CRGO
Agency ID: CRGO Period: 01 SEP 87 to 31 AUG 90

Objectives: Proj. 8700241. Cotesia congregata is a braconid wasp parasite of the tobacco hornworm, Manduca sexta. The female parasite injects a double-stranded DNA virus into the host larva, along with eggs, during parasitization of the host. A novel 33 kd polypeptide appears in the hemolymph within 2 to 4 hours post-parasitization and is induced in unparasitized larvae by the injection of virus isolated from the ovarian calyces of Cotesia. Thus this protein plausibly represents a viral gene product or a host gene product induced by the virus in the absence of parasites.

Approach: The goals of this project are to examine the temporal pattern of viral gene expression and characterize viral gene products; and to determine the amino acid sequence of the 33 kd polypeptide with the intent of assessing whether the gene which encodes for it lies within the host, parasite, or viral genome(s). Its regulation can then be studied under conditions of normal development of the wasps versus parasite encapsulation. Standard protein sequencing techniques will be used to obtain a partial sequence of the protein, then the likely DNA sequence encoding for the protein can be deduced. Viral sequences will be cloned and used for screening host tissues for viral replication.

Progress: 88/01 to 88/12. The braconid wasp Cotesia congregata injects a double-stranded DNA virus (polydnavirus) into host tobacco hornworm larvae, along with parasite eggs which develop in the hemocoel. In the past year we have purified and characterized this polydnavirus. Its genome is comprised of 17-24 double-stranded DNA circles ranging in size from 4 to >20 kb. Viral structural polypeptides were characterized using polyacrylamide gel electrophoresis. Northern blotting experiments are underway to clarify the temporal pattern of viral gene expression in the host, along with characterizing the role of viral gene products in modulating the host-parasite interaction. Detailed hemolymph polypeptide studies were carried out to characterize parasitism-specific polypeptides in naturally parasitized larvae, for comparison to proteins produced in unparasitized larvae injected with purified Cotesia polydnavirus and controls injected with inactivated virus. Both 1-D and 2-D gel analyses were employed, along with C4 reverse phase HPLC, to characterize hemolymph proteins. Our goal is to determine if the virus alone may be exploited as a biological pesticide to cause death of pest insects, circumventing the need for utilization of the parasites themselves. Development of parasite teratocytes, which are cells arising from the dissociation of the serosal cell layer encasing the embryo, was characterized using SEM, TEM, and flourescence activated cell sorter analyses.

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Publications: 88/01 to 88/12

BECKAGE, N.E., METCALF, J.S., NIELSON, B.D. and NESBIT, D.J. 1988. Disruptive effects of azadirachtin on development of Cotesia congregata in host tobacco hornworm larvae. Arch. Insect Biochem. Physiol. 9:47-65.

BECKAGE, N.E., NESBIT, D.J., NIELSEN, B.D., SPENSE, K.D. and BARMAN, M.A.E. 1988. Parasitic alteration of hemolymph polypeptides in Manduca sexta: a two-dimensional electrophoretic analysis and comparison.

THOMPSON, S.N., LEE, R.W.R. and BECKAGE, N.E. 1988. Metabolism of parasitized Manduca sexta examined by nuclear magnetic resonance. Arch. Insect Biochem. Physiol. In press.

THOMPSON, S.N., LEE, R.W.K. and BECKAGE, N.E. 1988. Characterization of the _3_1P spectrum Manduca sexta and effects of antimetabolites. Insect Biochem. In press.

BECKAGE, N.E., METCALF, J.S., NESBIT, D.J., SCHLEIFER, K.W. and ZETLAN, S.R. 1988. Parasitic inhibition of insect host hemolymph monophenoloxidase. Submitted to PNAS.

BECKAGE, N.E. 1988. Parasitic effects on host development. Chapter for UCLA Symposium Series book entitled New Directions in Biological Control. In preparation (to be submitted January, 1989.)

SCHLEIFER, K.S. 1988. Characterization of the genome and viral polypeptides of Cotesia congregata polydnavirus, and evidence for transcription of viral genes.

67.001* CRIS0136124
MOLECULAR APPROACH TO A GENE CONFERRING NEMATODE RESISTANCE TO TOMATO

WILLIAMSON V M; Nematology; University of California, Davis, **CALIFORNIA** 95616.
Proj. No.: CA-D*-NEM-5001-CG Project Type: CRGO
Agency ID: CRGO Period: 01 JUL 88 to 30 JUN 91

Objectives: PROJ. 8800667. Mi is a dominant locus that confers resistance to root-knot nematodes when present in tomato. Our goal is to clone Mi to increase understanding of the mechanism of resistance conferred by this gene.

Approach: Mi is closely linked to Aps-1, encoding acid phosphatase-1 in tomato. Acid phosphatase-1 will be purified from cell suspension culture and used to produce antibody and obtain peptide sequence for identification of the corresponding cDNA. Using this clone and clones of other linked fragments (RFLPs) as probes, DNA from resistant and susceptible cultivars will be analyzed. Candidate clones of Mi will be obtained by "chromosome walking" techniques. We propose to identify Mi by complementation of function after transformation of susceptible tomato cultivars with candidate clones using Agrobacterium based vectors.

Progress: 88/08 to 88/12. We have obtained data that will be of value in our attempt to clone the nematode resistance gene Mi of tomato by chromosome walking from the linked gene Aps-1. Using DNA clones which flank the region of Chromosome VI, which carries Aps-1 and Mi, 7 and 11 genetic map units away, we examined DNA from various tomato cultivars. Southern blot analyses were carried out using DNA from tomato cultivars that differ in the Aps-1 alleles, and in whether they carried Mi. Our results indicate that the size of the region of the tomato genome derived from the wild tomato species L. peruvainum (the source of Mi) varies among cultivars. This region of DNA is quite extensive in some cultivars, where it includes a DNA marker that is 7 map units away from Aps-1. This information will help us to localize the region of DNA encoding Mi for our chromosome walking experiments.

Publications: 88/08 to 88/12
No publications reported this period.

67.002* CRIS0130942
IMPROVING THE EFFICACY OF BACULOVIRUS PESTICIDES BY RECOMBINANT DNA TECHNOLOGY

MILLER L K; Entomology; University of Georgia, Athens, **GEORGIA** 30602.
Proj. No.: GEO-RC293-110 Project Type: CRGO
Agency ID: CRGO Period: 01 OCT 86 to 30 SEP 87

Objectives: PROJECT 8603163. Improve the efficacy of insect baculoviruses as biological pesticides by introducing insect behavior-modifying genes into baculoviruses. Use recombinant DNA technology to genetically construct a baculovirus that expresses a foreign gene which affects insect behavior.

Approach: Construct recombinant baculoviruses that carry a gene encoding an insect-specific neurotoxin and produce large quantities of toxin in infected insect cells. Determine if the recombinant virus is a more effective biological pesticide and if it has an extended host-range. Study possible strategies for reducing recombinant virus persistance in the environment to enhance the ecological safety of the pesticides.

Progress: 86/10 to 87/09. A gene encoding an insect-specific neurotoxin of scorpion venom, the Buthus eupeus insectotoxin I gene (BeIt1), has been synthesized from oligoneucleotides based on the published amino acid sequence of the toxin. The synthetic gene was cloned in E. coli and the sequence of the gene was confirmed by DNA sequencing. The BeIt1 gene was then transferred to an E. coli gene expression vector utilizing the Tac promoter to drive expression. No toxin expression was observed above background endotoxin activity in E. coli. The BeIt1 gene was transferred to a baculovirus expression system utilizing the polyhedrin promoter to drive toxin expression. No toxin activity was observed in recombinant BeIT1 baculovirus-infected cells. Toxin gene expression in infected insect cells was monitored at the protein synthesis level by pulse-labeling proteins with radioactive methionine. Only very low levels of a new 3.7 kilodalton protein was observed in the recombinant virus-infected cells. It is likely that the BeIT1 toxin is highly unstable under the conditions of expression used and if this gene is to be used to enhance baculovirus pesticide efficacy, a means of stabilizing the small polypeptide will need to be developed. The safety of recombinant baculoviruses with respect to mammalian species was also investigated during the course of this work.

Publications: 86/10 to 87/09
CARBONELL, L.F. and MILLER, L.K. 1987. Appl. Environ. Microbioly, 53:1412-1417. Baculovirus interaction with nontarget organisms: A virus-borne reporter gene is not expressed in two mammalian cell lines.
CARBONELL, L.F. and MILLER, L.K. 1987. Genetic engineering of viral pesticides: Expression of foreign genes in nonpermissive cells. In "Molecular Strategies for Crop Protection".
MILLER, L.K. 1987. Expression of foreign genes in insect cells. In "Biotechnology Advances in Invertebrate Pathology and Cell Culture" (ed. K. Maramorosch). Academic Press, Orlando, Fl.

67.003* CRIS0134015
REGULATION OF EXPRESSION OF THE BACULOVIRUS, ACNPV

FRIESEN P D; Bacteriology & Biochemistry; University of Idaho, Moscow, **IDAHO** 83843.
Proj. No.: IDA00908 Project Type: HATCH
Agency ID: CSRS Period: 01 JAN 88 to 06 JAN 89

Objectives: The long term objective of this proposal is to genetically engineer the baculoviruses for improved efficacy as

biological control agents of insect pests. Our immediate goals are to: determine the organization of several early viral genes for the insertion of foreign genes, locate those DNA sequences responsible for early and regulated expression of such genes, and examine the function of the early genes determing whether they are nonessential and are therefore replaceable. This proposal also examines the nature and mutagenic effects of an insect-derived transposable element which has integrated into the DNA genome of the baculovirus, AcNPV. Transposable elements act to decrease the virus' ability to produce its occluded form thereby reducing viral pesticide effectiveness.

Approach: Viral gene organization and the location of DNA control regions will be analyzed by fusing specific genes to easily assayed reported genes and testing them for proper expression in transient assays and by placing them back into the viral genome. Antibodies raised to trihybrid viral fusion proteins will be used to examine viral gene function. The gene organization and mutagenic effects of the transposable element will be determined by DNA sequence analysis and nucleic acid hybridization techniques.

Progress: 88/01 to 88/12. Progress in our investigation of the molecular mechanisms involved in the regulation of baculovirus gene expression has been the identification of DNA sequences responsible for conferring early and late transcription of the gene encoding a 35,000 - molecular - weight protein (35K) in the HindIII-K genome region of Autographa californica nuclear polyhedrosis virus (AcNPV). Mutagenesis of the 35K gene promoter previously linked to the reporter gene for chloramphenicol acetyl-transferase, indicated that sequences from -155 to -55 relative to the RNA start site (position +I) controlled early transcription while sequences from -55 to -4 controlled late transcription in recombinant viruses. Thus, two distinct regions of the 35K gene promoter are involved in early versus late regulation. Both regions contain sequences found at the promoter of other AcNPV genes with similar regulation and may therefore represent common control sequences. These studies on the nature of AcNPV promoters provide necessary information for the construction of recombinant baculoviruses expressing insecticidal genes for improved biological control of insect pests.

Publications: 88/01 to 88/12

NISSEN, M.S., and FRIESEN, P.D., (1989). Molecular Analysis of the Transcriptional Regulatory Region of Early Baculovirus Gene. J. Virology (in press).

67.004* CRIS0083485

ORGANIZATION AND EXPRESSION OF A BACULOVIRUS DNA GENOME

MILLER L K; Bacteriology & Biochemistry; University of Idaho, Moscow, **IDAHO** 83843.
Proj. No.: IDA00801 Project Type: HATCH
Agency ID: CSRS Period: 01 JUL 84 to 30 JUN 89

Objectives: The long-term objectives of this proposal are to improve the efficacy of viral pesticides by genetic engineering technology and facilitate the commercial production of these viruses. To achieve these objectives, more information conce ning viral gene organization and the regulation of gene expression is required. The immediate goals are, therefore, to locate key viral genes with respect to a physical map of the DNA genome, test an early promoter for controlling early expression of a passenger gene, and define the nature of genes involved in controlling gene expression. Since a handicap in the commercial production of viruses in cell culture was traced to the insertion of mobile genetic elements into the viral DNA.

Approach: Key genes of the baculovirus AcNPV will be mapped with respect to the established physical map of the viral DNA by marker rescue and by cloning cDNAs of early and intermediate viral mRNAs. Detailed knowledge of the nature of one such gene will be obtained; its promoter will be fused to an easily assayable gene and tested for temporal regulation. The position preferences of transposable element insertions will be determined and their effect on viral gene expression will be determined by nucleic acid hybridization techniques.

Progress: 86/01 to 86/12. Research has progressed in several different areas relevant to baculovirus gene orgainzation and expression that are applicable to development of more effective viral pesticides. First, we have synthesized and cloned complementary DNA from 20 different regions of the viral genome. Temporal expression of RNA from each region was examined and all were found to contain overlapping sets of RNA. Many of these overlapping sets of RNA have common 5' or common 3' termini, a common motif in the organization and expression of baculovirus genes. These studies are important since an understanding of the regulation of viral expression is required before the virus can be successfully engineered (via recombinant DNA) as improved pesticides. Secondly, to better understand this regulation, we have analyzed the structure of viral DNA during the viral replication cycle. We found that the viral genome adapts a nucleosomal-like structure typical of DNA undergoing active transcription. Thirdly, we have continued studies on the molecular biology of a transposable element (TED) which inserted into the baculovirus genome causing mutations. This led to the discovery that TED is a member of a newly characterized class of mutagenic elements which resemble the RNA tumor viruses.

Publications: 86/01 to 86/12

WILSON, J. and MILLER, L.K. 1986. Changes in the nucleoprotein complexes of a baculovirus DNA during infection. Virology 151:315-328.

MAINPRIZE, T.M., LEE, K.-J. and MILLER, L.K. 1986. Variation in temporal expression of overlapping baculovirus transcripts. Virus Res. 6:85-89.

FRIESEN, P.D., RICE, W.C., MILLER, D.W. and MILLER, L.K. 1986. Bidirectional transcription from a solo long terminal

repeat of the retrotransposon Ted: Symmetrical RNA start sites. Mol. Cell. Biol. 6:1599-1607.

67.005 0058005
CHROMATOGRAPHY OF BIOLOGICALLY IMPORTANT MOLECULES AND APPLICATIONS

GEHRKE C W; Biochemistry; University of Missouri, **MISSOURI** 65211.
Proj. No.: MO-00009 Project Type: HATCH
Agency ID: CSRS Period: 01 JUL 85 to 30 SEP 89

Objectives: Our research will center on DNA methylation and the m C content of human DNA sequences transcribed to different extents, and whether m C is actually found in dinucleotides other than m CpG and to examine other thermophilic organisms to determine their N -methylcytosine content. Research will be continued on advancement of high resolution HPLC for 60 modified nucleosides in tRNA and the structural characterization by UV and mass spectrometry of representative and unknown modified nucleosides. Studies will be made on chromatography and characterization of cap structures in mRNA and investigations on development of high resolution separation of RNA and DNA oligomers after base specific enzymatic hydrolysis.

Approach: Our approach is the advancement and further development of high resolution HPLC methods, multiwavelength UV spectral analysis, and interfaced capillary GC-MS for the accurate measurement and characterization of a wide array of major and modified nucleosides in DNA, tRNA, mRNA, oligomers; and for the development of pattern recognition studies by HPLC of enzymatic hydrolysates of peptides and proteins.

Progress: 88/01 to 88/12. We report standard RPLC-UV methologies for the analysis of more than 65 nucleosides in a single run with "run to run" peak retention variations of less than 1%. A complete nucleoside composition can be achieved with less than 0.5 microgram of RNA. Complete chromatographic protocols, nucleoside columns and parameters of operation are given for high resolution, high speed, and high sensitivity chromatography. Three unfractionated tRNAs are given as sources of reference compounds, also an extended enzymatic hydrolysis method. We are the first to report the presence of phosphorylated P-Ribosyl Adenosine in T-(psi)-Stem of yeast methionine initiator tRNA with characterization by HPLC-UV, MS, and NMR. Examples are given of unique applications of our advanced nucleoside methodology to biochemical and biomedical investigations including codon-anticodon discrimination and tRNA structural content a new method for quantitation of nucleosides in serum, and the reduced genomic content of m C in colonic neoplasias. These new research tools will have important contributions to biochemical and medical research.

Publications: 88/01 to 88/12

MCENTIRE, J.E., KUO, K.C., SMITH, M.E., STALLING, D.L., RICHENS, J.W. JR., ZUMWALT, R.W., FEHRKE, C.W., and PARPERMASTER, B.W.: Classification of Lung Cancer Patients and Controls by Chromatography of Modified Nucleosides in Serum.
KUO, K.C., PHAN, D.T., WILLIAMS, N., and GEHRKE, C.W.: Ribonucleosides in Serum and Urine by High Resolution Quantitative RPLC-UV Method. J. Chromatogr., in press.
PAPERMASTER, B.W., MCENTIRE, J.E., SMITH, M.E., BROWNSON, R.C., RICHENS, J.W. JR., KUO, K.C., and GEHRKE, C.W.: Increased Lung Cancer Mortality in a Rural Lead and Zinc Mining area: A Multivariate, Case-Control Study of Personal History Vi.
FEINBERG, A.P., GEHRKE, C.W., KUO, K.C. and EHRLICH, M.: Reduced Genomic 5-Methylcytosine Content in Human Colonic Neoplasis. Cancer Research 48:1159:1161, (1988).

CM 68 ANIMALS VERTEBRATES - NOT COMMODITY ORIENTED

68.001 CRIS0031648
REGULATION OF LIPOGENIC ENZYME AND APOLIPOPROTEIN LEVELS BY DIETARY CARBOHYDRATE AND LIPIDS

HOLTEN D D; Biochemistry; University of California, Riverside, **CALIFORNIA** 92521.
Proj. No.: CA-R*-BCH-2555 Project Type: STATE
Agency ID: SAES Period: 13 APR 88 to 30 SEP 92

Objectives: Determine to what extent transcription, translation and mRNA stabilization may be responsible for induction of these lipogenic enzymes; clone and sequence a full-length cDNA for G6PD; and use recombinant DNA technology to identify sequences in the G6PD mRNA or gene which are responsible for the regulation of G6PD synthesis.

Approach: Glucose-6-P dehydrogenase (G6PD) and 6-P-gluconate dehydrogenase (6PGD) are excellent models for using recombinant DNA technology to determine basic mechanisms by which dietary carbohydrate and fat interact with insulin, cAMP and glucocorticoids to regulate the synthesis of proteins involved in lipid metabolism.

Progress: 87/01 to 87/12. In live rats, liver glucose-6-P dehydrogenase synthesis and activity increases sixty-fold in response to fasting and refeeding a high carbohydrate diet. We have recently optimized conditions for culturing primary hepatocytes (isolated from fasted rats) in the absence of serum, hormones, carbohydrate or fat (1). Various hormones and carbohydrates were used to identify signals regulating G6PD synthesis. Conditions were found where G6PD synthesis increased 60-fold, mimicking changes seen in the live rat. Quantitation of G6PD mRNA was achieved by hybridizing Northern blots with a single stranded P cRNA probe for G6PD. In live rats or in hepatocytes cultured in a modified L15 medium lacking serum, hormones, carbohydrate or fat, G6PD mRNA and synthesis increased 10-15 fold within four days. Insulin increased G6PD synthesis an additional five-fold without any further increase in G6PD mRNA. Our results suggest that insulin regulates G6PD synthesis at the translational level. However, the approximately fifteen-fold increase in G6PD mRNA seems to occur without any requirement for hormones or carbohydrate. Polyunsaturated fatty acids (2) or glucagon may be responsible for the repression of G6PD synthesis and mRNA which is seen in live, fasted rats but not in hapatocytes cultured in the absence of these agents.

Publications: 87/01 to 87/12
MANOS, P. and HOLTEN, D. 1987. Primary cultures of hepatocytes in serum and hormone-free medium: Identification of conditions which stimulate an in vivo-like induction of G6PD. In Vitro 23:367-373.
TOMLINSON, J.E., NAKAYAMA, R. and HOLTEN, D. 1988. Repression of pentose phosphate pathway dehydrogenase synthesis and mRNA by dietary fat. J. Nutrition, in press.

68.002 CRIS0086322
MOLECULAR BASIS FOR GROWTH HORMONE GENE REGULATION

SPINDLER S R; Biochemistry; University of California, Riverside, **CALIFORNIA** 92521.
Proj. No.: CA-R*-BCH-4184 Project Type: STATE
Agency ID: SAES Period: 13 APR 88 to 30 SEP 92

Objectives: Determine the structure and function of the triiodothyronine inhibited and stimulated transcription-regulatory elements of the growth hormone gene. Physically map the transcription factor binding sites on these elements; structurally and functionally analyze the thyroid hormone receptor using our receptor gDNA and cDNA clones and the regulatory elements we have identified.

Approach: The structure and function studies of the regulatory elements will utilize in vitro mutagenesis and gene transfer. Sites of physical interaction between transcription factors and the regulatory elements will be mapped and characterized by in vitro footprinting and by in vivo DMS protection and genomic sequencing. The thyroid hormone receptor clones will be sequenced and functionally analyzed in vivo and in vitro using Northern blots and footprinting.

Progress: 87/01 to 87/12. We have previously shown that transcription of the rGH gene is transiently and cyclically induced by T3; identified the minimum region of DNA required for efficient, accurate and regulated transcription of the rat growth hormone gene; shown that thyroid hormone stimulates transcription of the gene at one discrete site, and inhibits transcription at another discrete site in this DNA region; determined the properties of each site; identified a thyroid-hormone-responsive chromatin domain located at each of these regulatory sites; and found a decrease in the level of growth hormone gene expression during aging. We will continue studies of these elements by: 1) structural and functional mapping of the triiodothyronine induced and inhibited regulatory regions; 2) physical mapping of transcription factor binding sites on these elements; 3) further cloning and structural-functional analysis of the rat %3 receptor gene(s) (c-erb-A genes).

Publications: 87/01 to 87/12
WIGHT, P.A., CREW, M.D. and SPINDLER, S.R. 1987. Discrete positive and negative thyroid hormone responsive transcription regulatory elements of the rat growth hormone gene. J. Biol. Chem. 262:5659-5662.
CREW, M.D., SPINDLER, S.R., WALFORD, R.L. and KOIZUMI, A. 1987. Age-related decrease in growth hormone and prolactin gene expression in the mouse pituitary. Endocrinology 121:1251-1255.
SPINDLER, S.R., CREW, M.D., WIGHT, P.A. and NYBORG, J.K. 1987. Thyroid hormone responsive regions of the growth hormone gene and chromatin. In, Mechanism of Action of Thyroid Hormone (DeGroot, Fradkin, Toleman, Eds.), Academic Press.
CREW, M.D., WIGHT, P.A. and SPINDLER, S.R. 1987. Thyroid hormone dependent transcriptional inhibition mediated by a 16

base pair element of the rat and human growth hormone genes which includes the TATA boxes. Cellular and Molecular Biol.
WIGHT, P.A., CREW, M.D. and SPINDLER, S.R. 1987. Sequences Essential for Activity of the Thyroid Hormone Responsive Transcription Stimulatory Element of the Rat Growth Hormone Gene. Molecular Endocrinology, submitted.
SPINDLER, S.R., KOIZUMI, A., WALFORD, R.L. and MOTE, P.L. 1988. Regulation of P1-450 and P3-450 Gene Expression and Maximum Life-Span are Related by Genes of the Major Histocompatibility Complex. Biochem. Biophys. Res. Commun., submitted.

68.003* CRIS0136409
SEX-SPECIFIC DNA IN LIVESTOCK ANIMALS

MCGRAW R A; College of Vet Medicine; University of Georgia, Athens, **GEORGIA** 30602.
Proj. No.: GEOV-0186 Project Type: STATE
Agency ID: CSVM Period: 01 JUL 87 to 30 JUN 91

Objectives: This project is aimed at identifying and characterizing sex-specific DNAs in economically important livestock species. The basic genetic information can be used to develop sex-specific DNA probes with potential application in assays for sex-fractionation of semen and/or sex determination of embryos.

Approach: The approach is to compare DNAs derived from male and female animals of each species by a variety of molecular genetic methods, including restriction analysis, cloning, sequencing, and hybridization techniques. DNA sequences unique to one of the sexes are then characterized and developed as sex-specific hybridization probes.

Progress: 87/07 to 88/12. This research is aimed at identifying and characterizing sex-specific DNAs in economically important livestock species. The genetic information is used to develop sex-specific DNA probes with potential application in assays for sex-fractionation of semen and in sex-identification of embryos. Methods include a variety of DNA manipulations: restriction enzyme digestions, electrophoretic separations, construction and propagation of recombinant DNA in bacteria, DNA sequece analysis, chemical DNA synthesis, enzymatic DNA amplification, and hybridizations using radioactively labelled probes. At this time, we have developed sex-specific probes in pigs and chickens. The procine probe has been used successfully for sex-identification of procine embryos and efforts are underway to attempt sex-fractionation of boar semen. Preliminary data suggests that we will be able to develop similar probes in horses and cattle.

Publications: 87/07 to 88/12
MCGRAW, R.A., JACOBSON, R.J. and AKAMATSU, M. 1988. A male-specific repeated DNA sequence in the domestic pig. Nucleic Acids Research 16(21):10389.

68.004 0032431
BIOCHEMISTRY OF ALDEHYDE METABOLISM

WEINER H; Biochemistry; Purdue University, West Lafayette, **INDIANA** 47907.
Proj. No.: IND053042 Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 84 to 30 SEP 89

Objectives: To study the enzymology, and molecular biology of aldehyde dehydrogenase; to study the metabolism of aldehyde and the effects of inhibition of aldehyde dehydrogenase on the process.

Approach: Chemical modification of purified liver and yeast aldehyde dehydrogenase will be performed. Metabolism will be studied in liver slices and possibly in hepatocytes. The effects of inhibiting specific isozymes in metabolism will be determined. In vitro translation of the mRNA coding for the enzyme will be performed and the relationship between the product formed and the isolated enzyme determined to see if precursors of the active enzyme exist. If they do their uptake into mitochondria will be studied. The yeast genes coding for the enzyme will be isolated, incorporated into plasmids and mutated so as to be able to study the roles of select amino acids in the enzyme.

Progress: 87/10 to 88/09. Much of our efforts have been directed at trying to elucidate the mechanism of action of liver aldehyde dehydrogenases, an enzyme involved in the detoxication of xenobiotics as well as endogenous aldehydes. To this end we have sequenced the cDNAs coding for the beef and rat liver mitochondrial enzymes and have initiated a project to study the yeast enzyme. We have been able to express and isolate active rat liver enzyme from E. coli. Having accomplished this it is now possible to employ site-directed mutagenesis to probe for the active site and other important domains of the enzyme. Thus far mutations at three cysteines and two glutamates are being investigated. The other major project under investigation is the import of pre-aldehyde dehydrogenase into mitochondria. We have been able to determine the amino acid sequence of the signal peptide portion of the enzyme necessary to allow for the import of the pre-enzyme into mitochondria. It was found that alcohol specifically inhibits import. The effects of alcohol and other components on the import process are being studied. Lastly, experiments are under way to isolate the membrane bound translocator.

Publications: 87/10 to 88/09
TU, C. C. and WEINER, H. (1988). Identification of the cysteine residue in the active site of horse liver mitochondrial. J. Biol. Chem. 263:1212-1217.
TU, G. C. and WEINER, H. (1988). Evidence for two distinct active sites on aldehyde dehydrogenase. J. Biol. Chem. 263:1218-1222.
FARRES, J. GUAN, K. L. and WEINER, H. (1988). Sequence of the signal for rat liver mitochondrial aldehyde dehydrogenase. Biochem. Biophys. Res. Commun. 150:1083-1087.

68.005* CRIS0077856
IMMUNOLOGY AND PATHOGENESIS OF PARASITIC DISEASES OF ANIMALS

KAZACOS K R; Veterinary Pathobiology; Purdue University, West Lafayette, **INDIANA** 47907.
Proj. No.: IND073029 Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 88 to 30 SEP 93

Objectives: For selected naturally occurring parasitic diseases of animals, determine: Immune responses, mechanisms, and immunodiagnosis. Pathogenesis, pathology, and parasitologic parameters. Prevalence and epidemiologic patterns. Focus these studies on the following diseases, and others as they might arise: Ascariasis (Ascaris suum) of swine. Trichinellosis (Trichinella spiralis of swine and wildlife. Nematode larva migrans diseases of animals (Baylisascaris, Tococara).

Approach: Naturally occurring and experimentally induced cases of selected parasitic diseases will be studied by laboratory and field observatios and methods, including the application of modern immunochemical and molecular biologic techniques.

Progress: 87/10 to 88/09. Twenty-one wildlife isolates of Trichinella spiralis were analyzed using DNA restriction fragment length polymorphisms (RFLPs). Similar RFLP patterns were seen with enzymes Eco RI, Hae III, Hpa II, Hind III and Xba I. Cla I digestion produced unique RFLPs indicating that the sylvatic group is a heterogeneous complex. Swine Trichinella RFLP was different from sylvatic Trichinella RFLPs. RFLP analysis and Southern blot hybridizations using a U.S.D.A. swine Trichinella-specific DNA probe confirmed that one coyote isolate actually represented sylvatically-maintained swine Trichinella. This has important implications for the epidemiology of swine trichinosis. Research on the immunodiagnosis of Baylisascaris larva migrans indicated the close relationship between B. melis and B. procyonis, based on gradient SDS-PAGE of larval excretory-secretory (ES) proteins. B. columnaris ES was less like the other two, and B. transfuga ES was most dissimilar. Carbohydrate staining and biotinylated lectin binding on Western blots indicated that B. procyonis ES antigens were complex glycoproteins with many sugars represented. Immunologic cross-reactivity was directed primarily at carbohydrate epitopes. Work was initiated to produce and characterize monoclonal antibodies against B. procyonis ES. Other research indicated that a portable flame gun is potentially useful for decreasing transmission of the swine roundworm Ascaris suum in growing-finishing swine units, through destruction of the resistant eggs.

Publications: 87/10 to 88/09

BOYCE, W.M., KAZACOS, E.O., KAZACOS, K.R. and ENGELHARDT, J.A. (1987). Pathology of pentastomid infections (Sebekia mississippiensis) in fish. J. Wildl. Dis. 23:689-692.

BOYCE, W.M., BRANSTETTER, B.A. and KAZACOS, K.R. (1988). In vitro culture of Baylisascaris procyonis and initial analysis of larval excretory-secretory antigens. Proc. Helminthol. Soc. Wash. 55:15-18.

KAZACOS, K.R. and KAZACOS, E.A. (1988). Diagnostic exercise: Neuromuscular condition in rabbits. Lab. Anim. Sci. 38:187-189.

BOYCE, W.M., BRANSTETTER, B.A., and KAZACOS, K.R. (1988). Comparative analysis ot larval excretory-secretory antigens of Baylisascaris procyonis, Toxocara canis and Ascaris suum by western blotting and enzyme immunoassay.

DIXON, D., REINHARD, G.R., KAZACOS, K.R., and ARRIAGA, C. (1988). Cerebrospinal nematodiasis in prairie dogs from a research facility. J. Am. Vet. Med. Assoc. 193:251-253.

HAMANN, K.J., KEPHART, G.M., KAZACOS, K.R., and GLEICH, G.J. Immunofluorescent localization of eosinophil granule major basic protein in fatal human cases of Baylisascaris procyonis infection. Am. J. Trop. Med. Hyg,. In press.

LITTLE, A.S. (1987). Immunological comparison of larval Baylisascaris procyonis, Toxocara canis, and Ascaris suum using immunodiffusion. M.S. Thesis, Purdue University, West Lafayette. 101 p.

68.006 CRIS0097798
EXPRESSION MODIFICATION OF A POSITION EFFECT MUTATION AT NOTCH IN DROSOPHILA

WELSHONS W J; Genetics; Iowa State University, Ames, **IOWA** 50011.
Proj. No.: IOW02759 Project Type: STATE
Agency ID: SAES Period: 01 JAN 86 to 31 DEC 90

Objectives: To identify genes closely linked to the Notch locus in Drosophila that modify the function of Notch in the presence of a small deletion at the distal end of the locus. We wish to find the linear order of these genes, their position on the polytene-X chromosome and estimate the maximum distance that can exist between Notch and the genes that modify function at Notch. Additionally, we will screen for genes elsewhere in the genome that modify the normal function of the Notch locus.

Approach: We will use chromosome aberrations that eliminate or enhance the functions that disturb normal activity at Notch. These chromosome disruptions will be characterized genetically, cytologically and molecularly. We will use enhancement or suppression of the mutant phenotype associated with the small deletion to identify other genes that interact with Notch in the development of the nervous system. When such genes are found, they can be cloned, characterized molecularly and their place in the developmental pathway of the nervous system can be recognized.

Progress: 88/01 to 88/12. A small chromosomal deletion called facet-strawberry (fa(superscript swb)) exerts a mutant effect upon the Notch (N) locus in Drosophila melanogaster although the deletion is outside the limits of the gene upon which it exerts its deleterious effect. This referral of the mutant

effect of fa(superscript swb) to the normal N gene is identified as a position effect. Experiments have demonstrated that the deleterious effects of the fa(superscript swb) referral to the Notch locus can be circumvented if chromosomal rearrangements are placed in a position immediately adjacent to the fa(superscript swb) deletion. Briefly stated: Given two closely linked genes that function simultaneously and independently, the function of one may interfere with the function of the other if the fa(superscript swb) deletion is placed between them. The deletion in fa(superscript swb) has eliminated DNA sequences that normally insulate the functions of one gene from the other, and if the function of the gene that interferes with N is eliminated by mutation, then N functions normally. Additional data have shown that if the gene that interferes with N becomes hyperactive, the mutant effect at N in enhanced.---We have analyzed a number of aberrations that alter the function of the gene that in turn interferes with N function.

Publications: 88/01 to 88/12

MARKOPOULOU, K., WELSHONS, W.J., and ARTAVANIS-TSAKONAS, S. (1989). Phenotypic and molecular analysis of the facets, a group of intronic mutations at the Notch locus of Drosophila melanogaster which affect postembryonic development.

68.007 CRIS0132081
DEVELOPMENT OF RECOMBINANT VACCINES FOR MYCOPLASMAL DISEASES

MINION F C; Veterinary Medical Research Institute; Iowa State University, Ames, **IOWA** 50011.
Proj. No.: IOWV-480-23-01 Project Type: STATE
Agency ID: CSVM Period: 01 NOV 86 to 31 OCT 90

Objectives: Analyze membrane activities of Mycoplasma pulmonis and M. gallisepticum which may be related to pathogenesis. Identify the proteins involved in these activities. Clone the genes which code for the proteins, construct and assess the efficacy of recombinant vaccines using these gene sequences.

Approach: Monoclonal antibodies will be used to identify the proteins of interest in M. pulmonis. Once developed, these antibodies will then be used to identify their corresponding gene sequences in chromosomal genes banks constructed in E. coli. These genes will then be altered so that their expression can be regulated to maximize expression for in vivo vaccine use.

Progress: 87/07 to 88/06. A recombinant DNA gene library for Mycoplasma pulmonis was constructed in a new Lambda gt11-like vector which will facilitate screening and mapping of cloned sequences. This library is being used to study gene regulation through the identification of mycoplasma specific antigenic proteins. Relevant DNA sequences will be subcloned, and the upstream promotor regions sequenced. In addition, methods have been developed to introduce both random and specific mutations in M. pulmonis using PEG-mediated transformation. Tn916 and Tn4001 were used to introduce random mutations and integrative plasmids based upon their antibiotic resistance elements used to introduce specific mutations. These systems have been used to establish homologous recombination in the Mollicutes as well as the discovery of a unique gene exchange mechanism operative in M. pulmonis. A number of marked strains have been produced and studies are underway to characterize them more fully. In other studies, monoclonal antibodies are being used to study membrane-associated activities and several have been identified which block hemolysis activity, a potential virulence factor. Using these antibodies, the protein responsible for hemolysis has been identified as an external 88.5 kDa membrane-bound protein.

Publications: 87/07 to 88/06

MAHAIRAS, G.G. and MINION, F.C. (1989). Random insertion of the gentamicin resistance transposon Tn4001 in Mycoplasma pulmonis. Plasmid. In Press.
MAHAIRAS, G.G. and MINION, F.C. (1989). Transformation of Mycoplasma pulmonis: Demonstration of homologous recombination, introduction of cloned genes and the preliminary description of an integrating shuttle system. J. Bacteriol.

68.008 CRIS0082436
FOOD POISONING TOXINS OF STAPHYLOCOCCUS AUREUS

IANDOLO J J; Biology; Kansas State University, Manhattan, **KANSAS** 66506.
Proj. No.: KAN00185 Project Type: STATE
Agency ID: SAES Period: 01 JUL 83 to 30 JUN 86

Objectives: The objectives of this work are to study the production of extracellular virulence factors of Staphylococcus aureus. In particular this work is directed towards understanding the food poisoning enterotoxins of these organisms and how they contribute to staphylococcal disease. The present work is directed toward unraveling the complex picture of production and extracellular elaboration. Although five toxins (enterotoxins A and B, Alpha-toxin, Beta-toxin and exfoliative toxin) will be studied, the work in this proposal will focus on enterotoxin B because of our extensive background with this species which therefore provides the best framework for constructing generalized models of expression.

Approach: By a blending of molecular, biochemical, genetic and animal toxicity testing I hope to provide a comprehensive picture of the regulation of toxin production and the transport of these substances to the extracellular environment. Ultimately, the results gathered from this work will be used to determine the significance of toxigenesis to the microbe and aid in later studies on the mode of action of the toxins in animal systems.

Progress: 80/07 to 86/06. The lipase gene of S. aureus (geh) was cloned and sequenced. The mechanism of bacteriophage regulation of the

enzymic activity was investigated and found to be due to insertion of the bacteriophage genome into the geh gene. Insertion occurred by homologous recombination between the phage att site and the bacterial att site. These sites consisted of an 18 bp region in the bacteriophage and the geh gene that are homologous. We also isolated the bacteriophage recombinase functions and were successful in inactivating geh by insertion of a plasmid containing the recombinase functions. The resistance determinants on the inserted plasmid were then used as a convenient marker to help in mapping the gene on the staphylococcal chromosome. We also successfully cloned and sequenced the exfoliative toxin A and B genes of S. aureus. These toxins have significant regions of homology that may prove useful in designing vaccines to protect against their activity. In vitro mutagenesis is being used to determine the location of the active site of he toxins. A new staphylococcal transposon Tn4291 which encodes for resistance to methicillin has been isolated and partially characterized. This transposon is approximately 7.1 kilobase pairs in size and exhibits site-specificity similar to Tn554. Further work is in progress to describe this element in more detail at the molecular level.

Publications: 80/07 to 86/06

DYER, D.W., ROCK, M.I., LEE, C.Y. and IANDOLO, J.J. 1985. Generation of transducing particles in Staphylococcus aureus. J. Bacteriol. 161: 91-95. KAES 85-82J.

CHRISTIANSON, K.K., TWETEN, R.K. and IANDOLO, J.J. Transport and processing of staphylococcal enterotoxin A. 1985. Applied and Environmental Microbiol. 50: 696-697. KAES 85-436J.

LEE, C.Y. and IANDOLO, J.J. 1985. Mechanism of bacteriophage conversion of lipase activity in Staphylococcus aureus. J. Bacteriol. 164: 288-293. KAES 85-462J.

LEE, C.Y. and IANDOLO, J.J. 1986. Lysogenic conversion of staphylococcal lipase is caused by insertion of the bacteriophage L54a genome into the lipase structural gene. J. Bacteriol. 166: 385-391. KAES 85-228J.

TWETEN, R.K. and IANDOLO, J.J. 1986. Purification of the staphylococcal enterotoxins. In R. Wu, Methods in Enzymology. In Press. KAES 86-259J.

JACKSON, M.P. and IANDOLO, J.J. 1986. Cloning and expression of the exfoliative toxin B gene from Staphylococcus aureus. J. Bacteriol. 166: 574-580. KAES 85-462J.

JACKSON, M.P. and IANDOLO, J.J. 1986. Sequence of the exfoliative toxin B gene of Staphylococcus aureus. J. Bacteriol. 167: 726-728. KAES 86-374J.

68.009 CRIS0098566

STUDIES OF STAPHYLOCOCCAL TOXINS

IANDOLO J J; Biology; Kansas State University, Manhattan, **KANSAS** 66506.

Proj. No.: KAN00623 Project Type: STATE
Agency ID: SAES Period: 01 JUL 86 to 30 JUN 89

Objectives: To study the production of extracellular virulence factors of Staphylococcus aureus. In particular this work is directed towards understanding the food poisoning enterotoxins and exfoliative toxins of these organisms and how they contribute to staphylococcal disease. The present work is directed toward unraveling the complex picture of production and extracellular elaboration. Although five toxins (enterotoxins A, B and C, and exfoliative toxin A and B) will be studied, the work in this proposal will focus on enterotoxin B because of our extensive background with this species which therefore provides the best framework for constructing generalized models of expression.

Approach: By a blending of molecular, biochemical, genetic and animal toxicity testing I hope to provide a comprehensive picture of the regulation and mechanism of action of these substances to the extracellular environment. Ultimately, the results gathered from this work will be used to determine the significance of toxigenesis to the microbe and aid in later studies on the mode of action of the toxins in animal systems.

Progress: 88/01 to 88/12. The lipase gene of S. aureus (geh) was cloned and sequenced. The mechanism of bacteriophage regulation of the enzymic activity was investigated and found to be due to insertion of the bacteriophage genome into the geh gene. Insertion occurred by homologous recombination between the phage att site and the bacterial att site. These sites consisted of an 18 bp region in the bacteriophage and the geh gene that are homologous. We also isolated the bacteriophage recombinase functions and were successful in activating geh by insertion of a plasmid containing the recombinase functions. The resistance determinants on inserted plasmid were then used as a convenient marker to help in mapping the gene on the staphylococcal chromosome. We also successfully cloned and sequenced the exfoliative toxin A and B genes of S. aureus. These toxins have significant regions of homology that may prove useful in designing vaccines to protect against their activity. In vitro mutagenesis is being used to determine the location of the active site of the toxins. A new staphlococcal transposon Tn4291 which encodes for resistance to methicillin has been isolated and partially characterized. This transposon is approximately 7.1 kilobase pairs in size and exhibits site-specificity similar to Tn554. Further work is in progress to describe this element in more detail at the molecular level.

Publications: 88/01 to 88/12

LEE, C.Y. and IANDOLO, J.J. 1988. Structural analysis of staphylococcal bacteriophage 11 attachment sites. J. Bacteriol. 170: 2409-2411.

TREES, D.L. and IANDOLO, J.J. 1988. Identification of a Staphylococcus aureus transposon (Tn4291) that carries the methicillin resistance gene(s). J. Bacteriol. 170: 149-154.

IANDOLO, J.J. 1988. The genetics ofstaphylococcal toxins and virulence factors. In Molecular basis of

pathogenicity. B.H. Iglewski and V.L. Clark, Eds. (KAES 88-271-B).
LIBBY, S. and IANDOLO, J.J. 1988. Genetics of methicillin resistance in Staphylococcus aureus. Assoc. for Prudent Use of Antibiotics (APUA) Newsletter (KAES 89-50-T).

68.010 CRIS0137745
MOLECULAR REGULATION OF STREPTOMYCES ANTIBIOTIC SYNTHESIS

CHAMPNESS W; Microbiology and Public Health; Michigan State University, East Lansing, **MICHIGAN** 48824.
Proj. No.: MICL01557 Project Type: HATCH - PENDING
Agency ID: CSRS Period: 01 APR 89 to 31 MAR 94

Objectives: The objective of this research is to define the molecular mechanisms for regulation of antibiotic synthesis in bacteria of the genus Streptomyces. This research will lead to increased capacity to engineer Streptomycetes for more efficient antibiotic production. The short term goals are to clone the newly discovered abs genes, which are likely to be regulators of antibiotic biosynthesis, to determine the regulation of expression of these genes and to determine the molecular mechanisms by which they affect antibiotic synthesis.

Approach: The problem of regulation of antibiotic synthesis has previously been approached, by other investigators, primarily from a physiological perspective. This research is taking the approach of using molecular genetics to discover the genes which control Streptomyces antibiotic synthesis and to understand how they function. We have already identified seveveral genes which are likely to be important components of the control pathway in the species S. coelicolor. Our analysis of these genes will include cloning, DNA sequencing and determination of transcriptional control signals. We will also determine whether these genes regulate antibiotic synthesis is all of the medically and agriculturally important Streptomycetes.

68.011 0058005
CHROMATOGRAPHY OF BIOLOGICALLY IMPORTANT MOLECULES AND APPLICATIONS

GEHRKE C W; Biochemistry; University of Missouri, **MISSOURI** 65211.
Proj. No.: MO-00009 Project Type: HATCH
Agency ID: CSRS Period: 01 JUL 85 to 30 SEP 89

Objectives: Our research will center on DNA methylation and the m C content of human DNA sequences transcribed to different extents, and whether m C is actually found in dinucleotides other than m CpG and to examine other thermophilic organisms to determine their N -methylcytosine content. Research will be continued on advancement of high resolution HPLC for 60 modified nucleosides in tRNA and the structural characterization by UV and mass spectrometry of representative and unknown modified nucleosides. Studies will be made on chromatography and characterization of cap structures in mRNA and investigations on development of high resolution separation of RNA and DNA oligomers after base specific enzymatic hydrolysis.

Approach: Our approach is the advancement and further development of high resolution HPLC methods, multiwavelength UV spectral analysis, and interfaced capillary GC-MS for the accurate measurement and characterization of a wide array of major and modified nucleosides in DNA, tRNA, mRNA, oligomers; and for the development of pattern recognition studies by HPLC of enzymatic hydrolysates of peptides and proteins.

Progress: 88/01 to 88/12. We report standard RPLC-UV methologies for the analysis of more than 65 nucleosides in a single run with "run to run" peak retention variations of less than 1%. A complete nucleoside composition can be achieved with less than 0.5 microgram of RNA. Complete chromatographic protocols, nucleoside columns and parameters of operation are given for high resolution, high speed, and high sensitivity chromatography. Three unfractionated tRNAs are given as sources of reference compounds, also an extended enzymatic hydrolysis method. We are the first to report the presence of phosphorylated P-Ribosyl Adenosine in T-(psi)-Stem of yeast methionine initiator tRNA with characterization by HPLC-UV, MS, and NMR. Examples are given of unique applications of our advanced nucleoside methodology to biochemical and biomedical investigations including codon-anticodon discrimination and tRNA structural content a new method for quantitation of nucleosides in serum, and the reduced genomic content of m C in colonic neoplasias. These new research tools will have important contributions to biochemical and medical research.

Publications: 88/01 to 88/12

MCENTIRE, J.E., KUO, K.C., SMITH, M.E., STALLING, D.L., RICHENS, J.W. JR., ZUMWALT, R.W., FEHRKE, C.W., and PARPERMASTER, B.W.: Classification of Lung Cancer Patients and Controls by Chromatography of Modified Nucleosides in Serum.
KUO, K.C., PHAN, D.T., WILLIAMS, N., and GEHRKE, C.W.: Ribonucleosides in Serum and Urine by High Resolution Quantitative RPLC-UV Method. J. Chromatogr., in press.
PAPERMASTER, B.W., MCENTIRE, J.E., SMITH, M.E., BROWNSON, R.C., RICHENS, J.W. JR., KUO, K.C., and GEHRKE, C.W.: Increased Lung Cancer Mortality in a Rural Lead and Zinc Mining area: A Multivariate, Case-Control Study of Personal History Vi.
FEINBERG, A.P., GEHRKE, C.W., KUO, K.C. and EHRLICH, M.: Reduced Genomic 5-Methylcytosine Content in Human Colonic Neoplasis. Cancer Research 48:1159:1161, (1988).

68.012* CRIS0098217
TOSCANA VIRUS EVOLUTION: IMPLICATIONS FOR USE OF VIRUS VACCINES AND DIAGNOSTIC PROBES

NICHOL S T; School of Veterinary Medicine; University of Nevada, Reno, **NEVADA** 89557.
Proj. No.: NEV00811 Project Type: ANIMAL HEALTH
Agency ID: CSRS Period: 01 JUL 86 to 30 JUN 89

Objectives: To determine the overall rate and content of genetic evaluation of Toscana virus during serial transovarial vertical transmission of the virus in sand flies. To ascertain which virus genes evolve most rapidly and which are highly conserved. To establish the exact sequence of virus genetic changes occurring during these serial infections.

Approach: Analyze evolution of Toscana virus in sand flies by T1 ribonuclease fingerprinting. Compare fingerprints of individual genome segments from each sand fly generation. Sequence RNA virus genomes of Toscana virus and compare with other RNA virus sequences using computer program.

Progress: 86/07 to 89/06. We have completed the sizing and T1 ribonuclease fingerprinting analysis of the RNA genomes of Toscana virus isolates from successive generations of an experimentally virus infected laboratory colony of Phlebotomus perniciosus sandflies. Virus genomes were found to consist of three genome RNA segments. An L segment of 6.5 Kb, and M segment of 4.4 Kb and an S segment of 2.1 Kb. No virus RNA genome changes were detected during transovarial transmission of the virus over 12 sandfly generations (a period of almost 2 years). These results demonstrate that although RNA viruses can exhibit high rates of mutational change under a variety of conditions, some virus RNA genomes are maintained in a stable manner during repeated transovarial virus transmission in the nautral insect host. The apparent stability may be due to the absence of host immune pressure and switching of host environment. This may allow the virus population to remain in a stable equilibrium weighted to the most fit virus. This finding has implications for the development of vaccines and diagnostic probes for insect transmitted RNA viral diseases of agricultural importance.

Publications: 86/07 to 89/06
NO PUBLICATIONS REPORTED THIS PERIOD.

68.013* CRIS0098768
TOSCANA VIRUS EVOLUTION: IMPLICATIONS FOR USE OF VIRUS VACCINES & DIAGNOSTIC PROBES

NICHOL S T; School of Veterinary Medicine; University of Nevada, Reno, **NEVADA** 89557.
Proj. No.: NEV00816 Project Type: HATCH
Agency ID: CSRS Period: 01 JUL 86 to 30 JUN 89

Objectives: To determine the overall rate and content of genetic evaluation of Toscana virus during serial transovarial vertical transmission of the virus in sand flies. To ascertain which virus genes evolve most rapidly and which are highly conserved. To establish the exact sequence of virus genetic changes occurring during these serial infections.

Approach: Analyze evolution of Toscana virus in sand flies by T1 ribonuclease fingerprinting. Compare fingerprints of individual genome segments from each sand fly generation. Sequence RNA virus genomes of Toscana virus and compare with other RNA virus sequences using computer program.

Progress: 88/01 to 88/12. We have completed the sizing and T1 ribonuclease fingerprinting analysis of the RNA genomes of Toscana virus isolates from successive generations of an experimentally virus infected laboratory colony of Phlebotomus perniciosus sandflies. Virus genomes were found to consist of three genome RNA segments. An L segment of 6.5 Kb, and M segment of 4.4 Kb and an S segment of 2.1 Kb. No virus RNA genome changes were detected during transovarial transmission of the virus over 12 sandfly generations (a period of almost 2 years). These results demonstrate that although RNA viruses can exhibit high rates of mutational change under a variety of conditions, some virus RNA genomes are maintained in a stable manner during repeated transovarial virus transmission in the natural insect host. The apparent stability may be due to the absence of host immune pressure and switching of host environment. This may allow the virus population to remain in a stable equilibrium weighted to the most fit virus. This finding has implications for the development of vaccines and diagnostic probes for insect transmitted RNA viral diseases of agricultural importance.

Publications: 88/01 to 88/12
NO PUBLICATIONS REPORTED THIS PERIOD.

68.014* CRIS0137894
CHARACTERIZATION OF THE MOLECULAR VARIABILITY OF VESICULAR STOMATITIS VIRUS

NICHOL S T; School of Veterinary Medicine; University of Nevada, Reno, **NEVADA** 89557.
Proj. No.: NEV00824 Project Type: HATCH
Agency ID: CSRS Period: 01 JUL 89 to 30 JUN 92

Objectives: To determine the nucleotide sequence of genes coding for the major antigens of eight vesicular stomatitis viruses.

Approach: The experimental approach to be employed includes analysis of the complete nucleotide sequence of the major antigen genes of at least 4 VSV NJ and IND isolates representative of each of the existing subtypes using the primer extension dideoxynucleotide sequencing technique. Computer analysis of the RNA and protein sequences using Microgenie and Ancstr software programs will allow the detailed comparison of these viruses.

68.015* CRIS0137911
CHARACTERIZATION OF THE MOLECULAR VARIABILITY OF VESICULAR STOMATITIS VIRUS

NICHOL S T; School of Veterinary Medicine; University of Nevada, Reno, **NEVADA** 89557.
Proj. No.: NEV00804 Project Type: ANIMAL HEALTH
Agency ID: CSRS Period: 01 JUL 89 to 30 JUN 92

Objectives: To determine the nucleotide sequence of genes coding for the major antigens of eight vesicular stomatitis viruses.

Approach: The experimental approach to be employed includes analysis of the complete nucleotide sequence of the major antigen genes of at least 4 VSV NJ and IND isolates representative of each of the existing subtypes using the primer extension dideoxynucleotide sequencing technique. Computer analysis of the RNA and protein sequences using Microgenie and Ancstr software programs will allow the detailed comparison of these viruses.

68.016* CRIS0099853
DEVELOPMENT OF AN EFFICIENT PROTEIN SECRETION SYSTEM IN STREPTOCOCCUS FAECALIS

DUNNY G M; WILSON D B; Microbiology; Cornell University, Ithaca, **NEW YORK** 14853.
Proj. No.: NYCV-433-323 Project Type: STATE
Agency ID: CSVM Period: 01 JUL 86 to 30 JUN 89

Objectives: The goal of this project is to develop a Streptococcus faecalis host-plasmid combination that causes the high level production and excretion of cloned genes.

Approach: The proposed research will focus on a protein called C130 that mediates a surface exclusion function in S. faecalis (expression of surface exclusion prevents donor-donor mating in a plasmid transfer system). In S. faecalis, induction of the synthesis of C130 results in its accumulation in the culture fluid. We propose to use recombinant DNA techniques to identify the portion of the C130 protein involved in secretion. We intend to maximize expression of this gene S. faecalis and to develop a system of fusing various foreign genes to the engineered derivative of C1300 in order to cause efficient production and secretion of important proteins.

Progress: 88/01 to 88/12. Our efforts focused on cloning and sequencing the plasmid-encoded gene encoding a protein, Tra 130 of enterococcus faecalis (Streptococcus faecalis) that would be used in the secretion system. We mapped the location of the structural gene, and have subconed the gene into sequencing vectors. Sequencing of the gne is currently in progress.

Publications: 88/01 to 88/12
CHRISTIE, P., KAO, S.-M., ADSIT, J., and DUNNY, G. 1988. Cloning and expression of genes encoding pheromone-inducible antigens of Enterococcus (Streptococcus) faecalis. J.Bacteriol. 170:5161-5168.

68.017* CRIS0097030
VACCINIA VIRUS: EUKARYOTIC CLONING AND EXPRESSION VECTOR

HRUBY D E; Microbiology; Oregon State University, Corvallis, **OREGON** 97331.
Proj. No.: ORE00034 Project Type: STATE
Agency ID: SAES Period: 15 SEP 85 to 14 SEP 90

Objectives: To develop improved methods of introducing foreign DNA into vaccinia virus. To use this methodology to construct recombinant viruses which may be used as vaccines or to produce bioreactive compounds.

Approach: DNA-mediated marker transfer techniques will be employed to insert genes of interest into vaccinia virus. Recombinant viruses will be isolated and characterized as to their genome structure, transcription of the foreign DNA, and expression of new proteins. The ability of recombinant viruses to induce appropriate immune reactions in vivo will be evaluated. Where indicated, the DNA sequence of the virus will be modified to improve the level of gene expression by the recombinant viruses.

Progress: 88/01 to 88/12. Vaccinia virus is being developed as an efficient system for the production of a variety of biopharmaceutical products as well as a recombinant vaccine vector. The experiments carried out in our laboratory at OSU are directed towards improving methods of recombinant virus construction and analyzing the biological properties of the imitant viruses that we are able to assemble. Results for the current year are: (1) Several regions of the Vv genome have been sequenced and subjected to rigorous molecular genetic analyses. The results of these experiments should provide information regarding both additional insertion sites for foreign genes and the location of viral promoter elements which can be used to express the passenger genetic information. (2) Genes encoding plant virus protease genes have been expressed in VV in a functional form. These vectors are currently being used to explore an experimental strategy for controlling the spread of plant pathogens in the field. (3) a recombinant vaccine against Streptococcus pyogenes has been developed. Further analysis of this recombinant may provide information which will allow the development of other vaccines against other animal and human pathogens which exhibit immunological diversity.

Publications: 88/01 to 88/12
HODGES, W.M. and HRUBY, D.E. (1987). Cell-free translation of a chimeric eukaryotic-prokaryotic message yields functional chloramphenicol acetyltransferase. Analytical Biochemistry 160:65-67.
WEINRICH, S.L. and HRUBY, D.E. (1987). Non-coordinate regulation of a vaccinia virus late gene cluster. J. Virol. 61:639-645.
FRANKE, C.A. and HRUBY, D.E. (1987). Association of non-viral proteins with recombinant vaccinia virus. Archives of Virology 94:347-351.

ROSEMAN, N.A. and HRUBY, D.E. (1987). Nucleotide sequence and transcript organization of a region of the vaccina virus genome which encodes a constitutively expressed gene required for DNA replication. J. Virol. 61:1398-1406.
FRANKE, C.A. and HRUBY, D.E. (1987). Quantitative assay of recombinant vaccinia virus-encoded neomycin phosphotransferase in infected eukaryotic cell lysates. J. Virol. Methods 16:147-154.
WILSON, E.M. and HRUBY, D.E. (1987). Use of single-stranded insertion vectors (SSIV) to introduce foreign genes into the vaccinia virus genome. Nuc. Acids. Res. 15:4690.
MINER, J., WEINRICH, S.L. and HRUBY, D.E. (1988). Molecular dissection if cis-acting regulatory elements from 5'-proximal regions of VV late gene cluster. J. Virol. 62:297-304.

68.018 CRIS0098770
INTERSPERSED REPETITIVE DNA IN THE RABBIT GENOME

HARDISON R C; Molecular and Cell Biology; Pennsylvania State University, University Park, **PENNSYLVANIA** 16802.
Proj. No.: PEN02881 Project Type: HATCH
Agency ID: CSRS Period: 01 JUL 86 to 30 JUN 90

Objectives: Determine the nucleotide sequence, copy number and transcriptional pattern for a long, interspersed repeat (L1) in the rabbit genome and analyze its transcription in heterologous cells.

Approach: Individual copies of the L1 repeat will be isolated by molecular cloning and the nucleotide sequence determined by dideoxynucleotide chain termination reactions. The copy number and transcriptional profile will be analyzed by hybridizing radioactive L1 DNA to rabbit genomic DNA or RNA, respectively. Introduction of cloned rabbit L1 DNA into mouse and human cells will allow analysis of the transcription of individual repeats without a background of heterogeneous transcripts.

Progress: 88/01 to 88/12. The rabbit genome contains two major types of repeated DNA, short elements called C repeats and long elements called L1 repeats. The C repeats are about 300 base pairs long, are transcribed by RNA polymerase III and are present in at least 200,000 copies per haploid genome. A full length L1 is 7500 base pairs long, although most copies are truncated. L1s are probably transcribed by RNA polymerase II and are present in about 50,000 copies per haploid genome. Both types of repeats are transposable elements, and they are proposed to move around the chromosomes via an RNA intermediate. The insertion of new copies of the repeats into some regions of the DNA can cause a loss of function, but many of the insertions have little effect. We recently discovered a positive effect of the insertion of some C repeats - they can provide a signal for polyadenylation of messenger RNA. We have shown that the L1 repeats have the capacity to encode two proteins, one of which is very large and is conserved in L1 repeats from other mammals. One intriguing result is that the two proteins are encoded in a dicistronic arrangement. This is not found in eukaryotic cellular genes, but it is found in some animal viruses. Hence we propose that the L1s are derived from viruses, and this helps to explain the odd distribution of L1s in various species - they may be transmitted horizontally as viruses.

Publications: 88/01 to 88/12
MARGOT, J.B., DEMERS, G.W., HARDISON, R.C. 1989. Complete nucleotide sequence of the rabbit beta-like globin gene cluster. J. Mol. Biol. in press.
DEMERS, G.W., MATUNIS, M.M., HARDISON, R.C. 1989. The L1 family of long, interspersed repetitive DNA in rabbits: Sequence, copy number, conserved open reading frames and homology to keratin. J. Mol. Evol. in press.
HARDISON, R., CHENG, J.F., DEMERS, G.W., MARGOT, J.B. 1987. Rabit alpha-like and beta-like globin gene clusters: Comparisons among mammalian globin gene clusters. in 'Developmental Control of Globin Gene Expression', eds. G.
Stamatoyannopoulos and A. Neinhais (A.R. Liss, NY) pp. 91-105.
DEMERS, G.W. 1987. L10c: A long interspersed repetitive DNA element in the rabbit genome. Ph.D. Thesis. Penn State University.

68.019 CRIS0133720
MITOCHONDRIAL GENES AND PLASMIDS IN MAMMALIAN CELLS

AVADHANI N G; Animal Biology; 3800 Spruce Street, Phildelphia, **PENNSYLVANIA** 19104.
Proj. No.: PENV-5-22921 Project Type: STATE
Agency ID: CSVM Period: 01 JUL 81 to 30 APR 86

Objectives: To understand the molecular and genetic regulatory mechanisms involved in the co-ordinate expression of a large number of nuclear genes coding for various mitochondrial proteins and a limited number of genes encoded in the mitochondrial genome.

Approach: To continue the studies on the mechanisms of nuclear-mitochondrial gene interactions as follows: certain cDNA clones will be selected and characterized by DNA sequence analysis; chromosomal location and organization of these genes will be determined in situ hybridization. Genes will be mapped and regulatory regions identified monospecific, high titer antibodies to mitochondrial genes will be developed and used to isolate the proteins by immune adsorption. These proteins will be micro injected into mouse fibroblast cells and ovocytes to study nuclear translocation and gene expression pattern; in vitro experiments will be carried out to determine the DNA binding properties of these proteins.

68.020 CRIS0136651
IDENTIFICATION OF DNA SEQUENCES REQUIRED TO TARGET PROTEINS TO THE MAMMARY GLAND

ROSEN J M; Baylor College of Medicine; One Baylor Plaza, Houston, **TEXAS** 77030.
Proj. No.: TEXR-8801477 Project Type: CRGO
Agency ID: CRGO Period: 01 SEP 88 to 31 AUG 91

Objectives: PROJ. 8801477. The long term goal of these studies is to identify cis-acting DNA sequences required to target specific genes to the mammary gland resulting in the efficient synthesis and secretion into milk of biologically important proteins.

Approach: We now propose to design a vector to target the efficient synthesis and secretion of any protein into milk of transgenic livestock. We will define more precisely the cis-acting DNA sequences in the immediate 5′ flanking region of the Beta-casein gene important to elicit mammary-specific expression and determine if these are capable of acting on a heterologous promoter. We will investigate the effects of enhancers, both those identified within the casein gene locus and heterologous enhancers, such as a glucocorticoid-response element, on the level of expression of the casein promoter-CAT fusion genes. We will define sequences within the Beta-casein gene that may be important for the accumulation of stable mRNA and the efficient synthesis and secretion of proteins in the mammary gland. We will search for dominant regulatory sequences, similar to those recently identified in the human Beta-globin locus, that can specify position independent expression of Beta-casein promoter-fusion genes.

68.021 CRIS0094590
CELLULAR AND MOLECULAR TOXICOLOGY

BRATTON G; BURGHARDT R; BUSBEE D; Veterinary Anatomy; Texas A&M University, College Station, **TEXAS** 77843.
Proj. No.: TEXV-0505 Project Type: STATE
Agency ID: CSVM Period: 02 NOV 84 to 31 DEC 92

Objectives: Gamete and preimplantation embryo physiology and genetic engineering. Bioactivation of mycotoxin aflatoxin B-1. Prenatal toxic and teratogenic symbols to identify hazardous agents associated with fossil fuels. Morphologic and metabolic responses of astrocytes in cattle and in culture to epliptogenic agents. Surface cables and surface coats of pathologic cardiac cells. Ontogony of oligodendroglia. EEpidermal implant union in percutaneous implants. The role of ion channel blockers in neurotoxicity. Membrane mechanisms of alcohol. Brain mechanisms of motor control. Marmoset wasting syndrome. Structural studies of the Bowhead whale and a marine stranding network. Behavioral evaluation of cat litter usage.

Approach: This project has been established to assist in animal health research capacity calculations according to section 1433, PL 95-113. This is a composite of molecular biology and cellular and molecular toxicology research projects which address models of human and animal disease for the purpose of contributing to human and animal health.

Progress: 88/01 to 88/12. This project assists in calculating animal health research capacity according to section 1433, PL95-113. The composite studies contribute to the understanding of normal and abnormal cells in both human and animal health. Nineteen projects were completed, 26 were continued, and three new ones were initiated. A list of projects by title is attached. Following is a summary of our findings in each of five general areas. Molecular and Cellular Toxicology: Toxins in fescue grass inhibit prolactin synthesis which reduces the formation of rough endoplasmic reticulum, thus reducing milk secretion in mammary ascinar cells. Broomweed has a direct toxic effect on whole embryos in culture that is modulated by GHS. Also, a method has been developed for thermally extracting chemical and drug residues from tissue; volatilizing them intact; separating them by intense chromatography; and quantitating them by line mass spectrometry. Cellular Neurobiology: Very low lead levels (less than 20 ug/dl blood) inhibit specific brain enzymes associated with myelination and induce morphological changes in astrocytes. Alcohol inhibits substance P synthesis and alters the function of several neurotransmitters in vivo and in vitro. Acute alcohol intake decreases cell membrane sialic acid in both the brain and liver. Comparative Genetics: The 21OH gene was mapped and RFLPs for 21OH, 17, and SSC were defined in cattle.

Publications: 88/01 to 88/12

NATION, J.R., FANTASIA, M.A., BRATTON, G.R. and WOMAC, C. Ethanol self-administration in rat following exposure to dietary cadmium. Neurotoxicology and Teratology 9:339-344, 1987.

NATION, J.R., WELLMAN, P.J., VON STULTZ, J., TAYLOR, B., CLARK, D.E. and BRATTON, G.R. Cadmium exposure results in decreased responsiveness to ethanol. Alcohol 5:99-102, 1988.

BRATTON, G.R. and KOWALCZYK, D.F. Lead poisoning. In: Current Therapy X (eds. R.W. Kirk et al.). W.B. Saunders, Co., Philadelphia. In press, 1988-1989.

NATION, J.R., FRYE, G.D., VON STULTZ, J. and BRATTON, G.R. Effects of combined lead and cadmium exposure: changes in schedule-controlled responding and in dopamine, serotonin, and their metabolites. Submitted to Behavioral Neuroscience.

BURGHARDT, R.C., KURTEN, D.G., BURGHARDT, R.L., KURTEN, R.C. and MITCHELL, P.A. Gap junction modulation in rat uterus. III. Structure-activity relationships of estrogen receptor-binding ligands on myometrial and serosal cells.

68.022 CRIS0093993
CHROMATIN STRUCTURE AND THE CONTROL OF GENE EXPRESSION IN MAMMALIAN CELLS

WIKTOROWICZ J E; Biochemistry & Nutrition; Virginia Poly Inst, Blacksburg, **VIRGINIA** 24061.
Proj. No.: VA-135058 Project Type: HATCH
Agency ID: CSRS Period: 01 OCT 84 to 30 SEP 89

Objectives: Using the established techniques of indirect end-labeling, the chromatin structure of active genes will be examined to: establish the existence of DNAase I and micrococcal nuclease hypersensitive sites within the gamma-globin gene and map their locations with respect to given restriction sites; examine the chromatin fine-structure with respect to the location of the proximal nucleosomes around the DNAase I hypersensitive site by concurrent digestion with exonuclease III; and determine and compare the mean nucleosome repeat lengths of bulk, gamma-globin flanking, and gamma-globin coding regions of K562 chromatin by digestion with micrococcal nuclease and by hybridization using homologous cloned sequences.

Approach: These objectives will be pursued using the techniques of recombinant DNA technology, tissue culture and analytical biochemistry. Tissue culture cells will be induced to synthesize hemoglobin, nuclei purified, and the globin gene chromatin structure analyzed by digestion with DNAase I, micrococcal nuclease, exonuclease III and hybridization with molecular clones of the globin genes.

Progress: 87/01 to 88/09. Clonal populations of human erythroleukemic K562 cells were exposed to sodium butyrate for 5 days. On the basis of hemoglobin production we isolated a number of phenotypically variable clones. These include a responsive clone (8B/b, 70-90% benzidine response) and an unresponsive clone (7E/a, 10-15% response), and a clone which synthesizes globin constituitively. Induction of the embryonic beta-like and adult alpha-like globins was observed for clone 8B/b, while gamma-globins, initially present at day 0, disappeared by day 5. Transcription of the gamma-globin gene in clone 7E/a remained constant at a low level, while total RNA synthesis increased, suggesting that globin expression in K562 cells is independent of total RNA synthesis. In clone 8B/b butyrate repressed transcription of the gamma-globin gene and total RNA, and the level of gamma-globin mRNA in the cytoplasm declined from its highest levels at day 0 to a minimum at day 5. The effect of butyrate in clone 8B/b, therefore, is to decrease the levels of fetal hemoglobin (alpha(2)gamma(2)) by decreasing the rate of gamma-globin transcription, while increasing the appearance of embryonic globins (alpha(2)epsilon(2), and zeta(2)eqsilon(2). Thus, induced 8B/b represents the state of globin gene expression in embryonic hematopoietic cells and may serve as a useful model in the study of the globin switching mechanism and in the study of control of gene expression in animals in general.

Publications: 87/01 to 88/09
NO PUBLICATIONS REPORTED THIS PERIOD.

68.023* CRIS0027332
METABOLISM AND MECHANISM OF ACTION OF VITAMIN D

DELUCA H F; SCHNOES H K; Biochemistry; University of Wisconsin, Madison, **WISCONSIN** 53706.
Proj. No.: WIS01114 Project Type: STATE
Agency ID: SAES Period: 01 JAN 60 to 30 JUN 90

Objectives: Delineate at the molecular level the metabolism and mechanism of action of vitamins A and D, and to determine hormones and nutritional factors which modulate their activity.

Approach: The primary approach is to synthesize radioactive vitamin A and vitamin D compounds, inject them into vitamin deficient animals, extract their tissues and determine by chromatographic methods the compounds to which they are converted. Once new metabolities are identified, they are isolated in pure form and their structures determined by mass spectrometry and nuclear magnetic resonance spectrometry. The structures of these compounds are then prepared by chemical synthesis and their biological activity determined in animals. Their use in agriculture and medicine are also tested when appropriate. The tissue conversions bringing about these compounds are searched for at the enzymatic or subcellular level; the enzymes are isolated and studied from a physical-chemical point of view. The mechanism of action of the final target hormone in the target tissue is determined by molecular biological techniques.

Progress: 88/01 to 88/12. The full length cDNA encoding for the entire 1,25-dihydroxyvitamin D(3) (1,25-(OH)(2)D(3)) rat receptor has been cloned, and its sequence determined. The deduced amino acid sequence agrees exactly with partial amino acid sequence obtained on pure isolated porcine intestinal 1,25-(OH)(2)D(3) receptor. The latter was obtained by means of an immunoaffinity column in which a monoclonal antibody against the porcine receptor was immobilized on Sepharose CL4B. The structure of the rat receptor is largely homologous to the reported human receptor structure in the DNA binding region and the ligand binding region. Continued attempts to demonstrate the presence of a 1,25-(OH)(2)D(3) responsive element in the calcium binding protein gene isolated from the rat has not met with success. By means of reporter genes we have probed the 5′ and 3′ ends of the calcium binding protein gene and the introns but none of these regions appear to have a sequence that results in stimulation of transcription of the CAT reporter system. We have developed a new chemical synthesis in which we can rapidly alter the side chain structure of 1,25-(OH)(2)D(3). We have prepared a large number of side chain analogs using this approach. Testing of some of them have revealed that by elongating the side chain in the 24-position, we can construct a compound that is active in causing differentiation but which

has no activity in mobilizing calcium.

Publications: 88/01 to 88/12

DARWISH, H.M., KRISINGER, J., STROM, M. and DELUCA, H.F. 1987. Molecular cloning of the cDNA and chromosomal gene for vitamin D-dependent calcium-binding protein of rat intestine. Proc. Natl. Acad. Sci. USA 84:6108-6111.

OSTREM, V.K., LAU, W.F., LEE, S.H., PERLMAN, K., PRAHL, J., SCHNOES, H.K., DELUCA, H.F. and IKEKAWA, N. 1987. Induction of monocytic differentiation of HL-60 cells by 1,25-dihydroxyvitamin D analogs. J. Biol. Chem. 262:14164-14171.

KUTNER, A., PERLMAN, K.L., LAGO, A., SCHNOES, H.K. and DELUCA, H.F. 1988. Novel convergent synthesis of side-chain-modified analogues of 1 alpha,25-dihydroxycholecalciferol and 1 alpha,25-dihydroxyergocalciferol.

DELUCA, H.F. 1988. The vitamin D story: A collaborative effort of basic science and clinical medicine. FASEB J. 2:224-236.

BURMESTER, J.K., MAEDA, N. and DELUCA, H.F. 1988. Isolation and expression of rat 1,25-dihydroxyvitamin D(3) receptor cDNA. Proc. Natl. Acad. Sci. USA 85:1005-1009.

BROWN, T.A., PRAHL, J.M. and DELUCA, H.F. 1988. Partial amino acid sequence of porcine 1,25-dihydroxyvitamin D(3) receptor isolated by immunoaffinity chromatography. Proc. Natl. Acad. Sci. USA 852454-2458.

DELUCA, H.F., SICINSKI, R.R., TANAKA, Y., STERN, P.H. and SMITH, C.M. 1988. Biological activity of 1,25-dihydroxyvitamin D(2) and 24-epi-1,25-dihydroxyvitamin D(2). Am. J. Physiol. 254:E402-E406.

68.024 CRIS0136648

BOVINE GENES CODING FOR PLACENTAL PROLACTIN-RELATED TRANSCRIPTS

SCHULER L A; Dept of Comparative Bioscience; 750 University Avenue, Madison, **WISCONSIN** 53706.

Proj. No.: WIS03251 Project Type: CRGO
Agency ID: CRGO Period: 01 SEP 88 to 31 AUG 91

Objectives: PROJ. 8801444. Identify and sequence remaining transcribed members of this gene family. Characterize representative genes with respect to nucleotide sequence-intron-exon junctions, exons, and flanking regions. Define transcription start sites. Describe chromosomal location.

Approach: Complete analysis of 180d cDNA library, investigate early gestational time points. Isolate genomic clones for bovine placental lactogen and bPRCI from bovine genomic library in Charon 28, map and sequence above elements. Use mouse-bovine somatic cell hybrids to identify chromosomal location, restrition map large genomic fragments once linkage has been established.

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